BIOLOGY OF SUBTERRANEAN POPULATIONS OF WOOLLY APPLE APHID, *ERIOSOMA LANIGERUM* (HAUSMANN) (HOMOPTERA: APHIDIDAE), IN APPLE ORCHARDS

By

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DECLARATION

I the undersigned hereby declare that the work contained in this dissertation is my own original work and I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: Date:

ABSTRACT

A study was made of the basic biology of subterranean *Eriosoma lanigerum* (Hausmann) populations in apple orchards in the Western Cape Province of South Africa.

The absence of cornicles and the presence of a vulva could be used to identify 1st instar nymphs and adults respectively. Body length, body width and length of the hind femur are useful for separating 2nd, 3rd and 4th instars. However, separation of 2nd from 3rd instar nymphs was very unreliable.

Maximum population growth rate was at 23 °C while at 30 °C population growth was zero. The estimated minimum and maximum threshold temperatures for development were 4.32 and more than 30 °C respectively.

Numbers of underground *E. lanigerum* in soil samples taken using mechanical and hand augers were similar. However, numbers of aphids in samples were influenced by the distance from the trunk at which the samples were taken and the presence and the type of root material in the samples. More aphids were recorded close to the trunk, and at a given distance from the trunk more aphids were recorded if there was root material in the sample, especially if the roots were galled.

early autumn (February, March) and declined during winter, especially if the winter rainfall was high. These cycles coincided with the nitrogen cycles in the roots.

Embryos were also present in all instars throughout the year. There were more embryos in the 4th instar and adult aphids than in the other instars. The highest number of embryos in the 4th instar and adult aphids occurred during spring, which coincided with peak nitrogen levels in the roots of apple trees. Nitrogen levels in root material adjacent to galls and in ungalled roots were higher than in root galls.

A number of entomopathogenic fungi, including species of *Conidiobolus*, *Hirsutella* and *Beauveria* were found. Their present contribution to biological control is not known.

A straw mulch suppressed subterranean *E. lanigerum* population levels at least as well as the soil insecticide, imidacloprid, currently in use.

OPSOMMING

'n Studie van die basiese biologie van ondergrondse bevolkings van *Eriosoma lanigerum* (Hausmann) is in appelboorde in die Weskaap Provinsie van Suid-Afrika uitgevoer.

Die afwesigheid van kornikels en die aanwesigheid van 'n vulva kon gebruik word om die 1ste instar nimfe en volwassennes onderskeidelik te identifiseer. Liggaamslengte, liggaamsbreedte en die lengte van die agterste femur kon gebruik word om die 2de, 3de en 4de instars van mekaar te onderskei. Onderskeiding tussen 2de en 3de instar nimfe was egter baie onbetroubaar.

Maksimum bevolkingsgroei het by 23 °C plaasgevind, terwyl dit nul was by 30 °C. Die beraamde minimum en maksimum temperature vir ontwikkeling was by 4.32 en meer as 30 °C onderskeidelik.

Getalle van ondergrondse *E. lanigerum* in grondmonsters wat geneem is met gebruik van meganiese en handbore was eenders. Getalle plantluise in monsters is egter beïnvloed deur die afstand vanaf die stam waarby die monsters geneem is en die teenwoordigheid van wortelmateriaal in die monsters. Meer plantluise is aangeteken as daar wortelmateriaal in die monsters was, en veral as daar galle op die wortels was.

Die appelbloedluis was dwarsdeur die jaar ondergronds aktief. Bevolkingsvlakke het gedurende die vroeë somer (November, Desember) en vroeë herfs (Februarie, Maart) toegeneem, en gedurende die winter afgeneem, veral as die winterreënval hoog was.

Embrio's was ook teenwoordig dwarsdeur die jaar. Daar was meer embrio's in die 4de instar en volwasse plantluise as in die ander instars. Die hoogste aantal embrio's in die 4de instar en volwasse plantluise het in die lente voorgekom, wat saamgeval het met piek stikstofvlakke in die wortels van appelbome. Stikstofvlakke in wortelmateriaal aangrensend aan wortels en in wortels sonder galle was hoër as in wortelgalle.

Talle entomopatogeniese swamme, insluitend spesies van *Conidiobolus*, *Hirsutella* en *Beauvaria* is gevind. Hulle huidige bydrae tot biologiese beheer is nie bekend nie.

'n Strooideklaag het ondergrondse bevolkingsvlakke van *E. lanigerum* tot ten minste dieselfde mate as die grondinsektedoder, imidacloprid, wat tans in gebruik is, onderdruk.

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CONTENTS

CHAPTER 1

INTRODUCTION

Eriosoma lanigerum (Hausmann) (Homoptera: Aphididae) is a native of eastern North America (Weber & Brown 1988) where it overwinters in the egg stage and passes one or two early spring generations on the white elm, *Ulmus americanus*, a tree that is not indigenous to South Africa (Annecke & Moran 1982). The limits of its distribution are between latitudes 64° N and 57° S, and longitudes 186°E and 159° 75' W (Asante 1994). It has been known as a pest of apple trees *Malus domestica* Borkh for nearly 200 years (Hoyt & Madson 1960). This plant originated from South East Asia. Therefore, the origin of the host plant (apple trees) and the pest (woolly apple aphid) is not the same. Today *E. lanigerum* is reported as a pest in commercial orchards and nursery plantings throughout the world (Walker *et al.* 1988). It feeds on shoots, branches, in pruning and other wounds, leaf axils and on the roots of apple trees. The first description of *E. lanigerum*, by Friedrich Hausmann, dates from 1802 (Baker 1915). The insect is covered with a secretion of wax, giving it a mealy appearance (Baker 1915).

The result of subterranean *E. lanigerum* feeding on apple tree roots is the formation of woody galls (Baker 1915), formed by cell hypertrophy and

hyperplasia, replacing normal xylem tissue and reducing the capacity of the roots to transport water and nutrients (Brown *et al.* 1991). In addition, populations of *E. lanigerum* feeding on roots of potted apple trees resulted in greater reduction in growth than populations feeding on the shoots (Weber & Brown 1988). However, despite the importance of damage caused by root-feeding *E. lanigerum* it has been more difficult to document and quantify than that caused by aerial populations because of difficulties in sampling subterranean aphids.

In South Africa *E. lanigerum* was first recorded in 1895 (Annecke & Moran 1982) and Fuller (1904) pointed out that it was found all over the country, and was the worst of all the pests, including fungi, attacking apples. In South Africa it is rarely found on other host plants, although it has been recorded on *Malus pumilus, Prunus domestica, Pyrus communis* (Millar 1994).

In the past the control of *E. lanigerum* has been based on the use of resistant rootstocks and chemical sprays. For instance, Fuller (1904), Staniland (1924), Knight *et al.* (1962), Rock & Zeiger (1974), Taylor (1981) and Young *et al.* (1982) have reported on apple rootstocks resistant to *E. lanigerum* and Myburgh (1962) suggested that the only permanent solution was in resistant rootstocks. Blommaert *et al.* (1968) recommended the use of

Merton 793 rootstocks in preference to the ordinary seedling rootstocks. However, Giliomee *et al.* (1968) reported that a strain of the aphid which had overcome the resistance factor in Northern Spy and related rootstocks had evolved in some areas of South Africa.

Fuller (1904) suggested that to control subterranean *E. lanigerum*, the surface soil should be removed and then nearly boiling hot water poured around the base of the tree, so as to saturate the soil well several inches deep. A further remedy was tobacco dust. Like hot water, it was applied after the surface soil had been scraped off, by sprinkling it around the base of the tree for a distance of two or three feet.

Myburgh & Van Niekerk (1964) found that that phorate had a remarkable effect on the below-ground *E. lanigerun* population and Blommaert *et al.* (1968) reported that phorate fluid proved very promising in preliminary trials. In 1962 a new systemic insecticide, vamidothion, was introduced for the control of aerial populations of woolly apple aphids (Tarr & Hyde-Wyatt 1965). This chemical was eventually widely used for the control of woolly apple aphid (Loubser 1968, Swart & Flight 1990). It was also used in a tank mixture with chlorpyrifos (Swart *et al.* 1992). However, tolerance to vamidothion in the Elgin area was later reported by Pringle *et al.* (1994).

Pringle (1998) showed that one application of imidacloprid controlled subterranean populations of *E. lanigerum* for at least three seasons. The good control of the root infesting populations resulted in greatly reduced aerial infestations. At present all orchards in the Elgin area not treated with imidacloprid require at least one spray application to control aerial population during summer.

No reports on the reproductive biology or seasonal population studies of subterranean *E. lanigerum* have been found, and reports of studies on *E. lanigerum* crawler movement between roots and aerial parts are limited (Bodenheimer 1947, Greenslade 1936, Hoyt & Madson 1960, and Lohrenz 1911). Therefore, a comprehensive investigation of the basic biology of *E. lanigerum* on the roots of apple trees was considered necessary as a basis for an integrated approach to the control of edaphic *E. lanigerum.*

The field studies were conducted on two farms in the Elgin area of the Western Cape Province. On the one farm, Oak Valley, a 4 ha orchard was used. This was divided into two blocks of 2 ha each. The orchard was planted in 1978 on seedling rootstocks and consisted of Granny Smith trees with some Golden Delicious pollinators. All sampling was carried out in the vicinity of the Granny Smith trees. Two orchards of approximately 2 ha each were used on the second farm, Molteno Trust. These orchards were planted in

1970 on M 793 rootstocks. Orchard 1 on Molteno Trust consisted of two rows of Granny Smith trees alternated with two rows of Golden Delicious trees. Sampling was conducted at the Granny Smith trees. Orchard 2 was a block of Starking trees and these were used for sampling. The necessity for irrigation was determined using neutron probes. Irrigation was applied from spitters 2 m a part which delivered a fine spray for a 1 m radius.

The research project focused specifically on the following aspects:

1. Identification of developmental stages of subterranean *E. lanigerum*;
2. A life table study of subterranean *E. lanigerum*;
3. The development of a system for the regular sampling of the subterranean *E. lanigerum* population;
4. Seasonal population fluctuation of the subterranean *E. lanigerum* population;
5. Breading cycles of *E. lanigerum* on the roots of apple trees;
6. Identification of natural enemies of subterranean *E. lanigerum*;
7. The possible use of a straw mulch for suppressing the subterranean population of *E. lanigerum*.

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CHAPTER 2

IDENTIFYING NYMPHAL INSTARS OF SUBTERRANEAN *ERIOSOMA LANIGERUM* (HAUSMANN)

Subterranean *Eriosoma lanigerum* (Hausmann), or woolly apple aphid, attacks the roots of apple trees, causing prominent galls which restrict the movement of water and nutrients (Brown *et al.* 1991). However, no studies have been conducted on this important component of the pest complex in South African apple orchards. The identification of the individual instars forms the basis of life history studies which, in turn, can be important in the planning of pest management strategies (Daly 1985). For example, certain developmental stages of arthropods are more susceptible to some mycopathogens than others (Susilo *et al.* 1994, Vacante *et al.* 1994).

Gautam & Verma (1987) indicated that antenna length, length of the first antennal segment and the length of the hind tarsus increased with each successive *E. lanigerum* instar on the aerial parts of the apple trees. Asante & Cairns (1995) found that the most important characters for separating the instars on the aerial parts of apple trees were distance between cornicles, cauda width, body length and hind tibia length.

No information on classifying subterranean *E. lanigerum* from South

African apple orchards is available. Therefore, the object of the present study was to use the measurements of a number of structures of these aphids to identify which structures can most reliably be used to identify the different instars.

2.1. MATERIAL AND METHODS

Specimens of subterranean *E. lanigerum* were collected from two sites, Oak Valley and Molteno Trust, every second week. On each site 60 trees were identified at the beginning of the study and on each sampling date, five trees were randomly selected from these. Infested roots were removed from the five trees and transported to the laboratory in a cool bag. If a tree did not have infested roots, roots were taken from a neighbouring tree in an adjacent row.

E. lanigerum specimens were washed off the roots into a sieve with a mesh size of 200μ (the methods used are described in Chapter 4). They were roughly categorised into instars based on the proboscis length relative to the body length (Baker 1915, Gautum & Verma 1987), the shape of the abdomen and the number of antennal segments, which increases from five to six after the first (Asante & Cairns 1995) or second moult (Gautum & Verma 1987) in the case of aerial *E. lanigerum*. A maximum of five individuals in each

developmental stage were used, except in the case of the 4th instar where ten individuals were measured from each site. The following structures were measured to within 1/100 mm, using an ocular micrometer eyepiece in a microscope: body length, body width, distance between the antenna, length of 3rd, 4th and 5th* antennal segments, distance between the eyes, length of the last segment of the proboscis, distance between the cornicles, cornicle diameter, cauda width, length of the 2nd segment of the hind tarsus and length of the hind femur. Body length was measured from the front of the head to the anal plate, while body width was measured at the widest part of the body. After measuring body length and width, the head was removed to measure the distances between eyes and antennae. The antennal segments, the last segment of the proboscis, the 2nd segment of the hind tarsus and the hind femur were separated and placed in glycerine under a cover slip before being measured.

The data were analysed using a factorial analysis of variance with developmental stage, farm and date as main effects. Significant probability levels for instars would indicate differences between instars in the structure in question, while significant probability levels for date or farm would indicate

* The division of the 3rd antennal segment into the 3rd and 4th segment resulted in the 4th antennal segment becoming the 5th antennal segment.

that the structure in question differed between dates or farms respectively and would, therefore, not be a suitable structure for identifying instars. In addition, interactions between the main effects would indicate that differences were not consistent between dates or between farms. Finite mixtures analysis (Flury 1995, Flury 1997) was used to determine whether or not the measurements of the above structures separated the aphids into distinct categories (instars), and discriminant analysis was used to quantify the degree of agreement between the original subjective classification and the classification produced using this multivariate model.

Cornicles only appear after the first moult, while the vulva is only present in adult females (Asante & Cairns 1995). The lack of cornicles was used to positively identify first instar nymphs and the presence of a vulva was used to identify adults. Therefore, these two developmental stages were omitted from the above analysis.

2.2 RESULTS AND DISCUSSION

There was an increase in the size of most of the structures measured with each successive developmental stage (Table 2.1). However, the 4th antennal segment showed a reduction in length between the 2nd and 3rd instars and between the 3rd and 4th instars. This was probably because it

appeared as a new 4th segment after the first (Asante & Cairns 1995) or second moult (Gautam & Verma 1987), when the 3rd antennal segment divided.

Table 2.1. Mean sizes (±SE) in millimeters of morphological structures of the developmental stages of subterranean *E. lanigerum*.

Structure	Instar 1	Instar 2	Instar 3	Instar 4	Adult
Body length	0.696(±0.006)	0.782(±0.004)	0.933(±0.007)	1.516(±0.016)	1.726(±0.017)
Body width	0.256(±0.002)	0.289(±0.002)	0.377(±0.003)	0.772(±0.011)	1.053(±0.009)
3rd antennal segment	0.063(±0.0005)	0.067(±0.0006)	0.073(±0.003)	0.098(±0.002)	0.127(±0.005)
4th antennal segment	0.053(±0.0005)	0.058(±0.002)	0.052(±0.0007)	0.046(±0.0005)	0.049(±0.001)
5th antennal segment			0.054(±0.001)	0.062(±0.0005)	0.069(±0.003)
Between eyes	0.158(±0.001)	0.165(±0.001)	0.182(±0.001)	0.262(±0.003)	0.312(±0.002)
Between antenna	0.123(±0.006)	0.117(±0.0009)	0.124(±0.001)	0.161(±0.001)	0.176(±0.001)
Cauda width	0.06(±0.0007)	0.063(±0.0005)	0.076(±0.002)	0.133(±0.002)	0.159(±0.002)
Between cornicles		0.138(±0.038)	0.234(±0.003)	0.411(±0.005)	0.522(±0.004)
Cornicle width		0.068(±0.054)	0.031(±0.0004)	0.053(±0.001)	0.066(±0.003)
Length of last proboscis segment	0.119(±0.0003)	0.122(±0.0006)	0.123(±0.0005)	0.142(±0.0007)	0.154(±0.0007)
length of 2nd segment of hind tarsus	0.069(±0.0003)	0.071(±0.0005)	0.071(±0.0004)	0.083(±0.002)	0.093(±0.005)
Hind femur length	0.155(±0.0008)	0.161(±0.001)	0.174(±0.001)	0.256(±0.003)	0.308(±0.002)

In the 3rd instar, 12.1% of the aphids had six antennal segments, 67.4% in the 4th instar and 78.5% of the adult aphids had six antennal segments. Therefore, division of the 3rd antennal segment did not consistently occur in a particular instar as reported by Gautam & Verma (1987) and Asante & Cairns (1995). In addition the distance between antennae in the 2nd instar decreased, while the length of the 2nd segment of the tarsus in the 2nd and the 3rd instars was the same (Table 2.1).

There were differences between instars, dates and farms ($P<0.05$) for most of the structures measured (Table 2.2). However, the distances between the eyes did not differ between instars, dates or farms indicating that this character would not be suitable for separating instars. Cornicle width and length of the 2nd segment of the tarsus did not differ between dates and farms but differed between instars, suggesting that they may be suitable characters for separating instars. In addition the length of the 4th antennal segment did not differ between farms but differed between dates and instars. Therefore, although the size of this structure differed between instars, it changed through the year (Table 2.2). The date X instar interactions for the length of the 4th antennal segment and the distances between the eyes were significant. However, interactions between other characters were not significant, indicating that differences between instars were the same on all dates except

Table 2.2. Probability levels for differences between date, instar and farm in the sizes of different structures of subterranean *E. lanigerum* and for interactions between these main effects.

STRUCTURE MEASURED	DATE	INSTAR	FARM	DATE x INSTAR	DATE x FARM	INSTAR x FARM	DATE x INSTAR x FARM
Body length	<0.001	<0.001	<0.001	0.979	0.883	0.003	0.999
Body width	0.01	<0.001	0.008	0.999	0.488	0.117	0.999
3rd antennal segment	<0.001	<0.001	0.008	0.952	0.598	0.336	0.493
4th antennal segment	0.008	<0.001	0.389	<0.001	0.747	0.117	0.005
5th antennal segment	<0.001	<0.001	0.006	0.969	0.059	0.586	0.733
Between eyes	0.411	0.380	0.268	0.033	0.426	0.261	0.033
Between antennae	<0.001	<0.001	<0.001	0.930	0.251	<0.001	0.985
Between cornicles	<0.001	<0.001	<0.001	0.999	0.472	0.333	0.999
Cornicle width	0.137	<0.001	0.08	0.991	0.583	0.498	0.992
Cauda width	0.03	<0.001	0.01	0.59	0.875	0.214	0.919
Hind femur length	<0.001	<0.001	<0.001	0.988	0.253	0.071	0.999
Length of 2nd hind tarsal segment	0.06	<0.001	0.632	0.958	0.892	0.94	1.00
Length of last proboscis segment	<0.001	<0.001	<0.001	0.874	0.04	0.359	0.999

for the 4th antennal segment and the distance between the eyes (Table 2.2). The date X farm interactions for proboscis length and the instar X farm interactions for the body length and the distance between antennae were significant. The date X instar X farm interactions for the 4th antennal segment and the distance between eyes were also significant (Table 2.2).

Convergence in the finite mixtures analysis when three categories (instars 2, 3 and 4) were entered was only achieved for three of the structures measured. These were body length, body width and length of hind femur. The results are given in Table 2.3.

In all three cases there was more overlap between the 2nd and 3rd than between the 3rd and 4th instars (Figs. 2.1, 2.2 and 2.3), suggesting that more erroneous classsifications would be made between the 2nd and 3rd than between the 3rd and 4th instars. In addition, the proportions of 2nd, 3rd and 4th instars obtained in the finite mixture analysis (Table 2.3) using the three structures were not the same, indicating that the classification into instars differed according to the structure used.

The lack of convergence in the case of the other structures measured indicated that using finite mixtures analysis three groups (instars) could not be identified. However, the analysis of variance indicated significant differences between instars for cornicle width and length of the second hind tarsal

segment (Table 2.2). There were no differences between farms or dates or interactions for these structures, suggesting that they are suitable structures for identification of instars. However, although cornicles were present in the 2nd instar they were often too small to be measured accurately. Plots of measurements of the length of the 2nd hind tarsal segment (Fig. 2.4) showed that there was a lot of overlap between the 2nd and 3rd instars. Therefore, the differences between instars indicated in Table 2.2 resulted from differences between the 4th and 3rd instars and between the 4th and 2nd instars, without any separation occurring between the 2nd and 3rd instars. This explains the lack of convergence in the finite mixture analysis.

Table 2.3. Proportion of aphids classified into instars 2, 3 and 4 with the average measurements and variances for body length, body width and length of hind femur (N = numbers measured).

Structure	Instar	Proportion	Average measurement	Variance
Body length	2	0.340	0.812	0.0081
N = 1031	3	0.405	1.117	0.0359
	4	0.253	1.796	0.0725
Body width	2	0.271	0.295	0.0008
N =1034	3	0.482	0.472	0.0132
	4	0.246	0.986	0.0205
Hind femur	2	0.384	0.167	0.1832
length	3	0.447	0.241	0.1477
N =988	4	0.168	0.378	0.2045

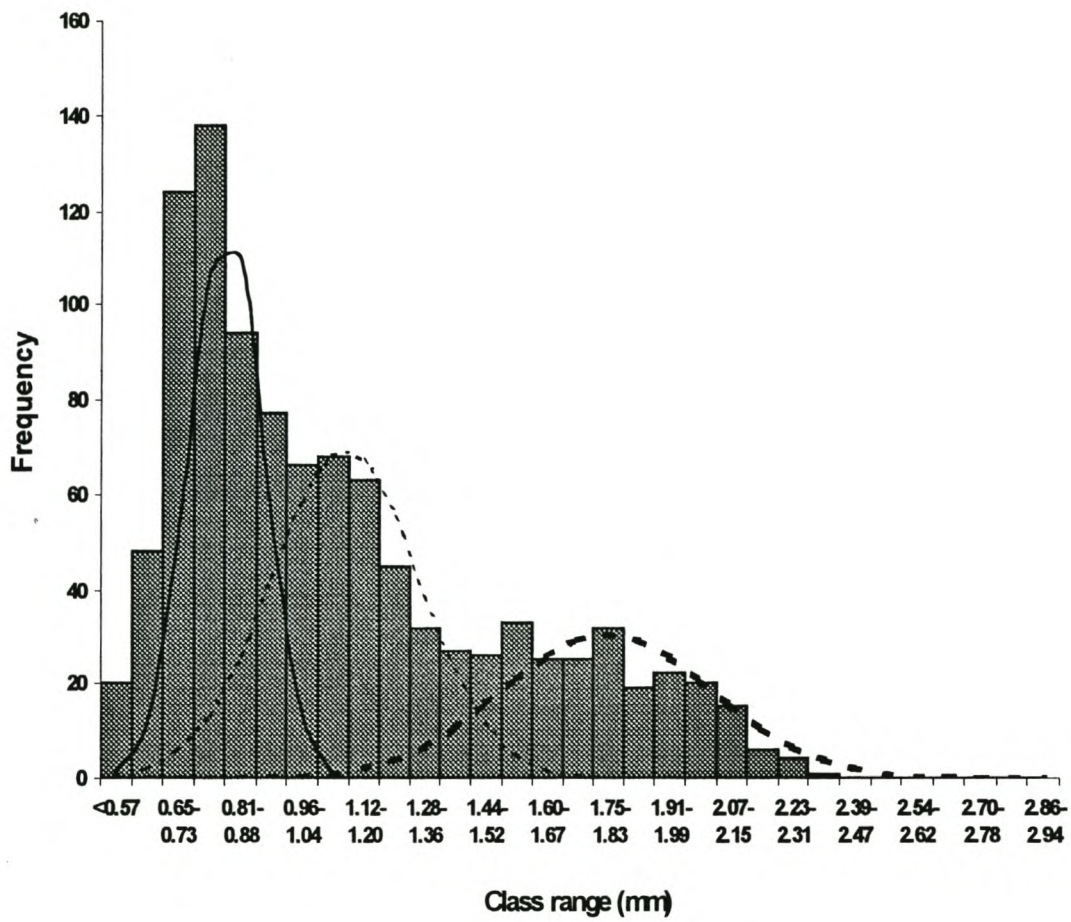

Fig. 2.1. Frequency distribution for measurements of *Eriosoma lanigerum* body length (bars), and estimated frequency distribution of instars 2, 3 and 4 obtained from finite mixture analysis. Thin solid line = 2nd instar; thin broken line = 3rd instar; thick broken line = 4th instar.

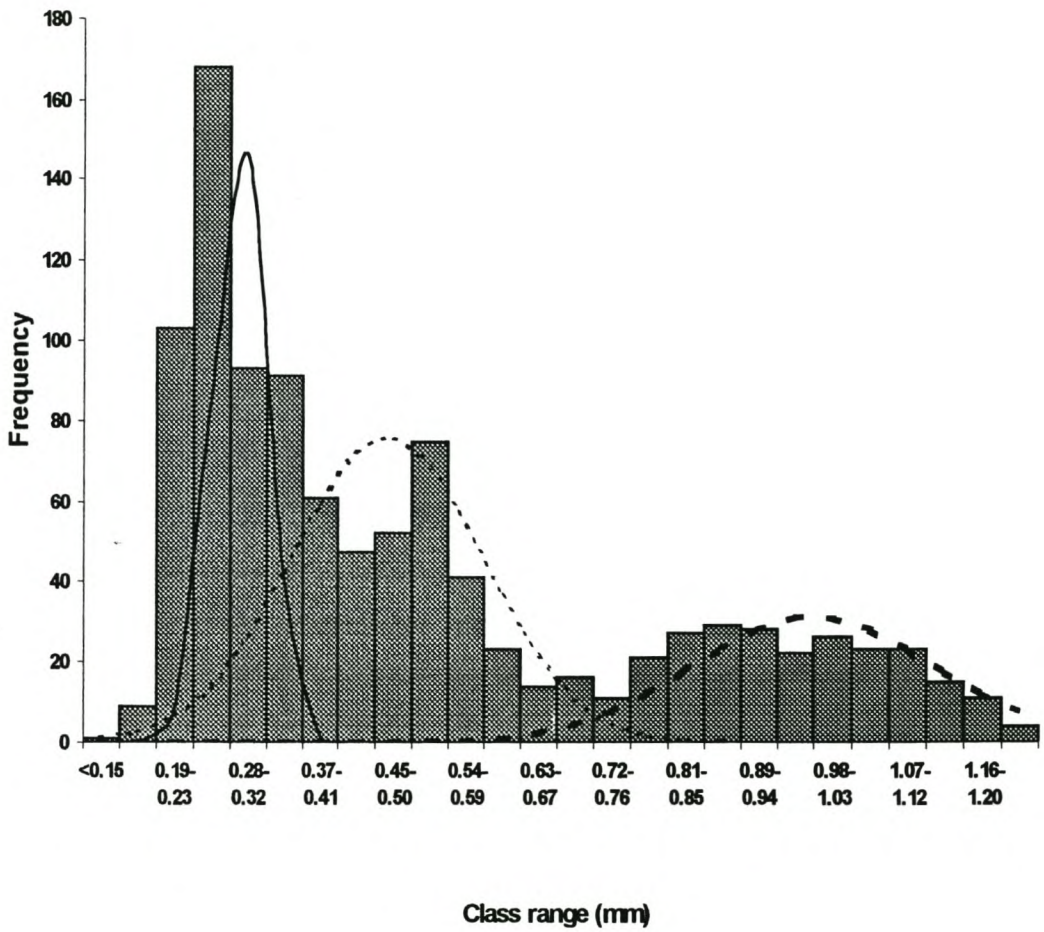

Fig. 2.2. Frequency distribution for measurements of *Eriosoma lanigerum* body width (bars), and estimated frequency distribution of instars 2, 3 and 4 obtained from finite mixture analysis. Thin solid line = 2nd instar; thin broken line = 3rd instar; thick broken line = 4th instar.

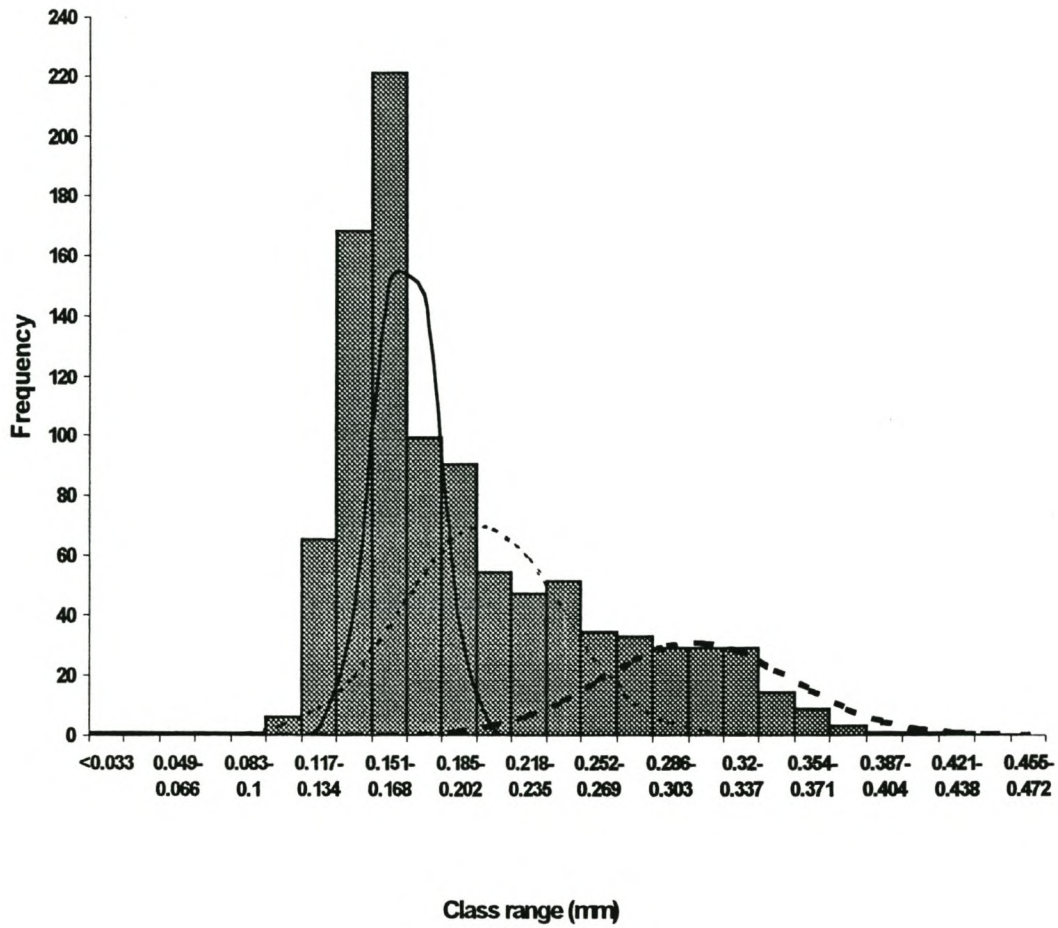

Fig. 2.3. Frequency distribution for measurements of *Eriosoma lanigerum* hind femur length (bars), and estimated frequency distribution of instars 2, 3 and 4 obtained from finite mixture analysis. Thin solid line = 2nd instar; thin broken line = 3rd instar; thick broken line = 4th instar.

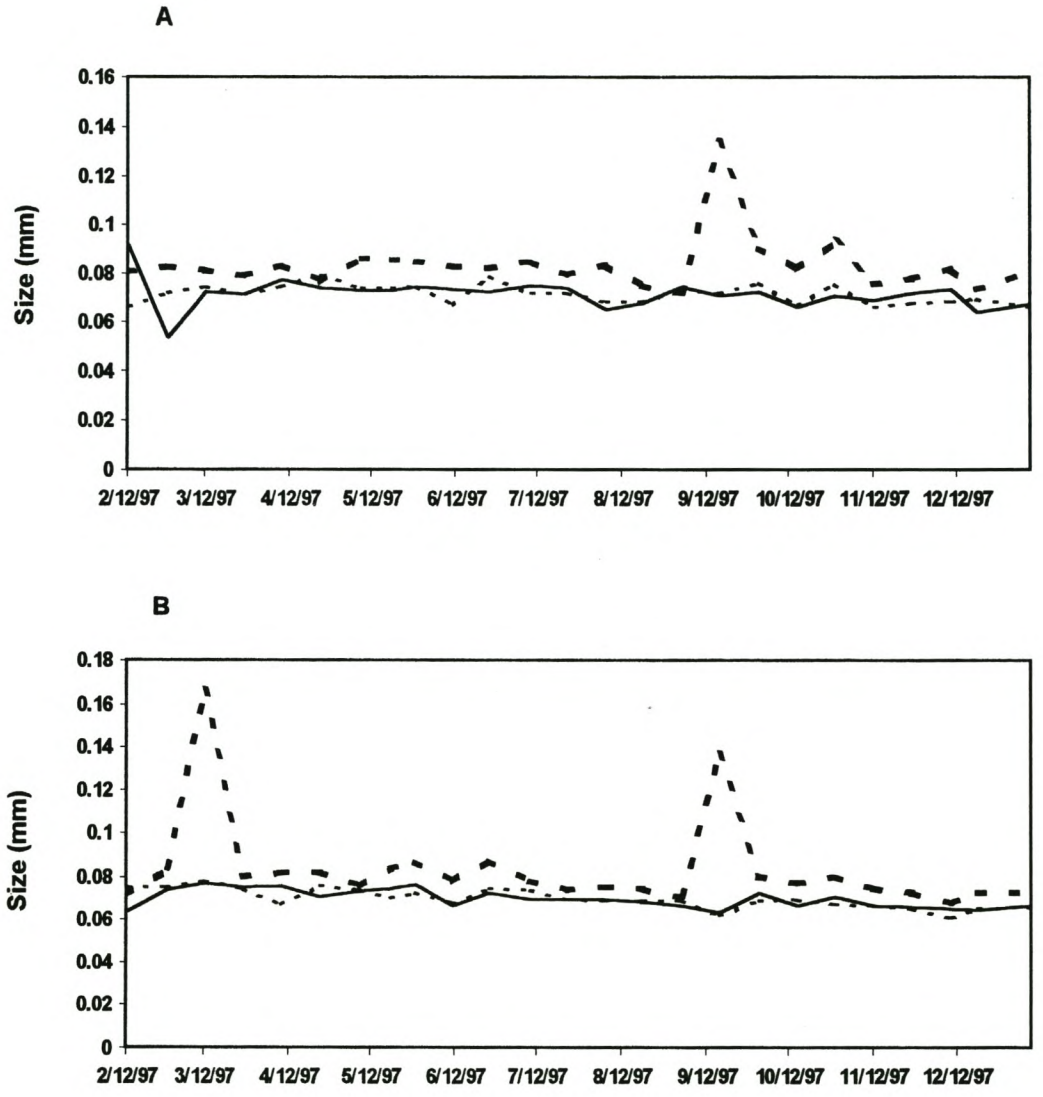

Fig. 2.4. Average length of the 2nd tarsal segment in the 2nd (thin solid line), 3rd (thin broken line) and 4th (thick broken line) instar on different dates during 1997. A, Oak Valley; B, Molteno Trust.

Table 2.4. F-values to enter (entr) or remove (rem) variables with their degrees of freedom and probability levels in the successive steps in the initial descriminant analysis.

Variables	Step	F to entr/rem	df 1	df 2	P-level
Body width	1	751.2805	2	940	0.000000
Body length	2	18.0758	2	939	0.000000
Last segment of the proboscis	3	8.598	2	938	0.000199
Between antenna	4	3.7165	2	937	0.024678
Third segment of the antenna	5	4.0006	2	936	0.018619
Between eyes	6	2.8654	2	935	0.057460
Cauda width	7	2.0654	2	934	0.127344
Hind femur length	8	1.9845	2	933	0.138033

In the initial discriminant analysis the first three variables to be entered were body length, body width and length of the last segment of the proboscis (Table 2.4). The latter was one of the characters for which convergence was not achieved in the finite mixtures analysis. Hind femur length, for which convergence was achieved, was the last factor to be entered into the stepwise discriminant function. However, when only body length, body width and hind femur length were used the probability to enter hind femur length was highly significant (Table 2.5). In addition, when body length, body width, length of the last segment of the proboscis and hind femur length were included 79.1% of

the classifications agreed with the original classification, and when length of the last segment of the proboscis was excluded this increased to 81.8% agreement with the original classification. Therefore, the three factors included in the discriminant function were body length, body width and hind femur length. A summary of this analysis is given in Table 2.5.

Table 2.5. F-values, degrees of freedom and probability to enter three characters into a stepwise discriminant analysis of characters required to classify 2nd, 3rd and 4th instars of subterranean *E. lanigerum.*

Variable	F	d.f.	P
Body width	751.2805	2, 940	< 0.001
Body length	18.0758	2, 939	< 0.001
Femur	6.6372	2, 938	0.0013

The relatively large standardised coefficients in the first discriminant function and the high correlation with this function for body length and body width (Table 2.6) indicate that the first discriminant function is strongly influenced by overall size of the nymphs. The large negative standardised coefficient and correlation with the second discriminant function for hind femur length indicated that this function represented hind femur length. The correlation between hind femur length and the first function was also high, but

the standardised coefficient was small.

The eigen value for the first discriminant function was 1.698 which represented 99.2% of the variance. Therefore, most of the separation was achieved using the first discriminant function. This is supported by the plot of the second versus the first discriminant function (Fig. 2.5) which shows greater separation between instars in the horizontal than in the vertical plane. This graph (Fig. 2.5) also shows that separation between instars 2 and 3 is less reliable than between instars 3 and 4 supporting the conclusions drawn from the finite mixture analysis. The constants for the two discriminant functions are given in Table 2.7.

Table. 2.6. Standardised coefficients and correlation of the characters with the two discriminant functions.

	Discriminant function 1		**Discriminant function 2**	
Characters	**Standardised**		**Standardised**	
	Coefficient	**Correlation**	**Coefficient**	**Correlation**
Body length	0.570	0.970	0.024	-0.140
Body width	0.544	0.969	1.363	0.042
Hind femur length	-0.101	0.802	-1.676	-0.564

2.3 CONCLUSION

The first instar can be identified by the absence of cornicles, and the adult by the presence of a vulva. Accurate separation between the 2nd and 3rd instar is not possible, as there is a large degree of overlap in the size of the most suitable structures that can be used (see Figs. 2.1, 2.2 and 2.3). In addition the discriminant function (Fig. 2.5) also indicates a large degree of overlap between instars 2 and 3. The first discriminant function can be used to separate instar 4 from 2 and 3. Body length is probably the single most useful character for separation of instar 2 from instar 3 as there is the least overlap in body length between these instars (compare Fig. 2.1 with Figs. 2.2 and 2.3). This is supported by the posterior probability curves relating body length, body width and hind femur length to the probability of classifying an aphid into instar 2, 3 or 4. (Figs. 2.6. A, B and C). The increasing probability of classifying an aphid as 3rd instar instead of 2nd instar with decreasing body length at the extreme left of the graphs reflects the high degree of overlap in body length, body width and hind femur length between 2nd and 3rd instars, as well as the relatively large variance (Table 2.3). This phenomenon is more obvious for body width and hind femur length (Fig. 2.6. B and C) than for body length.

The discriminant analysis was based on a subjective initial classification. However. The results were supported by the finite mixtures analysis, which was not dependent on an initial classification, but the finite mixtures analysis used was univariate. Therefore, these characters can not be combined in a single classification model using this finite mixtures analysis.

Table 2.7. Classification function for the three characters required to classify 2nd, 3rd and 4th instars of subterranean *E. lanigerum.*

Structure	Instar 2	Instar 3	Instar 4
Body width	-44.753	-42.8582	-35.4144
Body length	18.8952	21.806	25.4955
Femur	146.1517	131.6541	134.182
Constant	-14.2477	-14.9587	-23.621

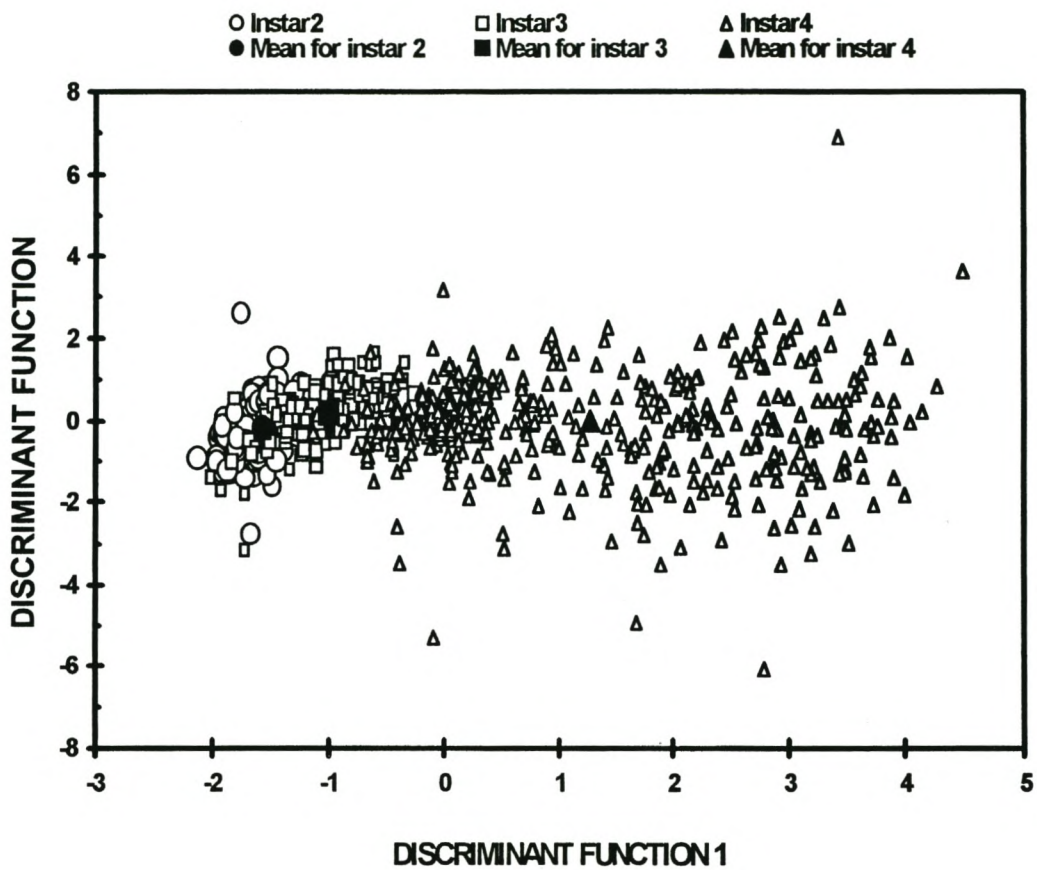

Fig. 2.5. Discriminant function 2 plotted against dicriminant function 1 for 2nd, 3rd and 4th instar field collected nymphs of subterranean *E. lanigerum*.

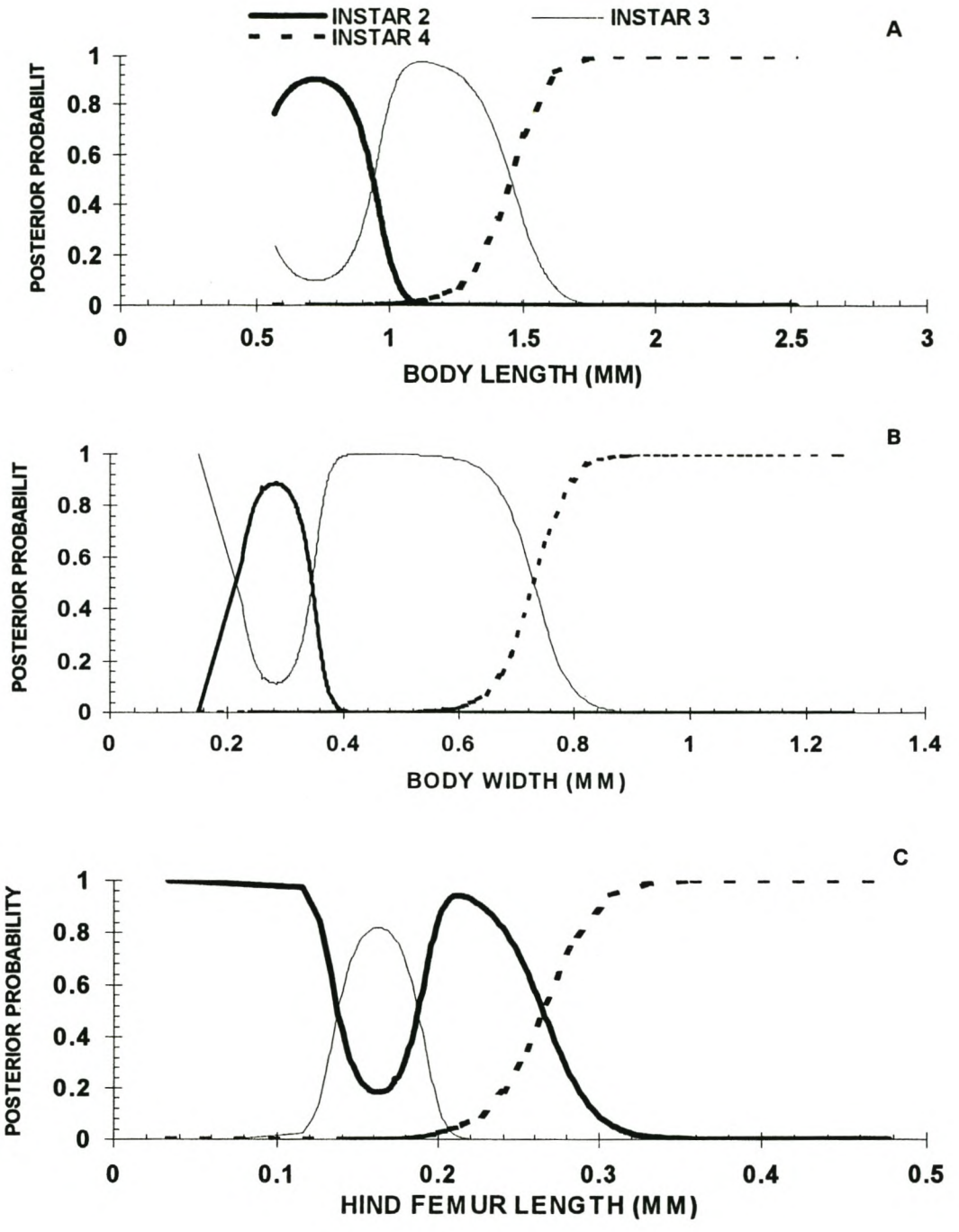

Fig.2.6. Posterior probability curves relating body width (A), body length (B) and hind femur length (C) to probability of classifying on aphid as instar 2, 3 or 4.

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CHAPTER 3

EFFECT OF CONSTANT TEMPERATURE ON DEVELOPMENT, GROWTH AND SURVIVAL OF SUBTERRANEAN *ERIOSOMA LANIGERUM*

Eriosoma lanigerum (Hausman) lives on the roots of apple trees where it forms galls, thereby reducing the ability of the roots to transport water and nutrients (Brown *et al.* 1991). In the Western Cape Province of South Africa high numbers of crawlers move from the roots up the trunk of the trees to the aerial parts during spring, where they damage the developing buds. No information is available on the reproductive capacity of these subterranean populations of *E. lanigerum,* and how temperature affects the rate of development and reproduction.

Therefore the aim of this life table study was to determine the optimum temperature for development of edaphic *E. lanigerum*, the range of temperatures at which it could remain active on the roots of apple trees and the number of degree days required to complete development.

3.1 MATERIAL AND METHODS

Roots and galls infested with *E. lanigerum* were placed on sterilized filter paper in sterilized petri dishes. Each petri dish contained about fifty

adults. Crawlers born during the following 24 hours were transferred to roots of one year old Royal Gala apple trees on seedling root stock in pots 12 cm high and 15 cm in diameter. The roots were not covered with soil as this would have made daily observations of the aphids impossible without disturbing them. The crawlers were placed on the exposed roots after which the top of each pot was covered with black plastic. The pots were placed into cooled incubators set at different temperatures. The temperatures used were 18, 20, 23, 27 and 30 °C. In many cases the crawlers fed and started to move around and sometimes continued this behaviour for several days before settling down. At 18 and 20 °C subterranean *E. lanigerum* crawlers at different stages of development moved around more than at other temperatures. The data from individual aphids were needed to determine rates of development, fecundity, the minimum and maximum threshold temperatures for development. Therefore, crawlers that had settled were isolated with a sticky material, Plantex®, consisting of a mixture of polybutene and wax. This prevented contact with other crawlers. If some aphids moved around at other temperatures, the data obtained from these individuals were not used in the calculations.

Infested trees were kept in the incubators at the appropriate temperature until the aphids had completed their development. Observations

on development, number of progeny and mortality were made 12 hourly at 20, 23, 27 and 30 °C and 24 hourly at 18 °C. The presence of exuviae indicated that individuals had moulted. The number of crawlers produced by each female was recorded until the females died. The net replacement rate (Ro) and mean generation time (T) were calculated as described by Price (1984). These values were then used to obtain an initial estimate of the intrinsic rate of increase (r_m) (Price 1984),

$$r_m = [\ln(R_0)]/T.$$

The estimates of r_m were then used in the first iteration to solve the equation (Watson 1964),

$$\sum_{x=1}^{t} e^{-r_m x} L_x M_x = 1;\ X = 1,2,3\ldots,\ \text{tdays}$$

where X = age interval in days, L_x = proportion of females alive at age X and M_x = mean number of crawlers produced by each aphid during the age interval X. Iteration was continued until the left-hand side of the equation was within 0.0001 of the right-hand side. It was not necessary to account for the sex ratio in the life table calculation as the subterranean *E. lanigerum* used were parthenogenic and all the progeny were females.

The reciprocal of time to complete development (in days) was regressed on temperature. The minimum temperature for development was

then estimated by solving the regression equation for 1/Time = 0. Data obtained at 30 °C were not used in these calculations as this temperature was stressful to the aphids (see discussion). The number degree-days (°D) required for development was calculated using °D = 1/b, where b is the slope of regression of 1/Time on temperature (Campbell *et al.* 1974).

The upper threshold temperature for optimum development was estimated by fitting a quadratic function of 1/Time on temperature to all the data, including 30 °C, setting the first derivative equal to zero and solving for X (temperature).

3.2 RESULTS

As the temperature increased, development time decreased (Table 3.1) except at 30 °C, while the generation time (T) and net replacement rate (R_o) decreased, including at 30 °C. The net replacement rate of 1 at 30 °C indicated that at this temperature there was zero population growth.

Survival was longest at 18 °C (Fig. 3.1) and decreased rapidly as the temperature increased. The intrinsic rate of natural increase, r_m, reached a peak at 23 °C. At 30 °C it was almost zero (Table 3.1), also indicating zero population growth at this temperature. Therefore, the upper threshold for population growth appears to be 30 °C. The estimated minimum threshold

temperature for development was 4.32 °C (Fig. 3.2) which was also the base for degree day calculations. The maximum rate of development was at 26.8 °C (Fig. 3.3), while the estimated number of °D required to complete development from crawler to adult was 291.12 °D. Although population growth did not occur at 30 °C, development from crawler to adult was completed. Therefore, the upper threshold temperature for development was above 30 °C.

Table 3.1. Average time in days for *E. lanigerum* to develop from crawler to adult on roots of potted apple trees (± standard deviation) at five different temperatures. n = number of aphids used; T = generation time; R_o = net replacement rate or the average number of crawlers produced per adult; r_m = intrinsic rate of natural increase.

Temperature (^{0}C)	Time (days)	T(days)	Ro	r_m
18	23.7 (± 4.6) (n = 20)	44.99	75.34	0.119
20	17.07 (± 2.97) (n = 14)	31.64	49.103	0.152
23	14.5 (± 1.03) (n = 28)	25.27	40.76	0.177
27	13.37 (± 1.74) (n = 19)	20.41	19.94	0.161
30	14.4 (± 2.7) (n = 25)	17.28	1	0.00001

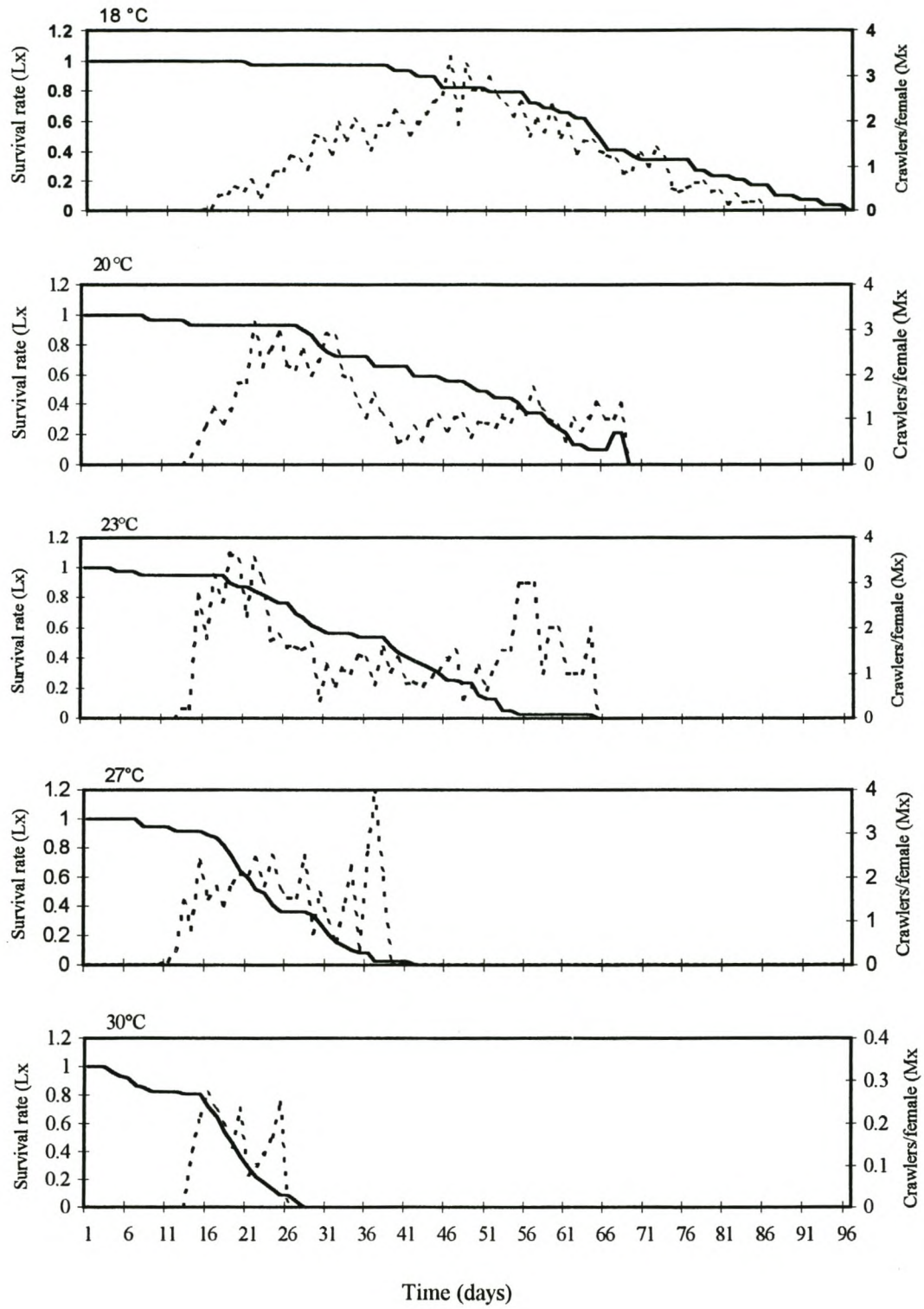

Fig.3.1. Survival, Lx, (solid line) and fecundity, Mx, (broken line) of subterranean *Eriosoma lanigerum* at five temperatures.

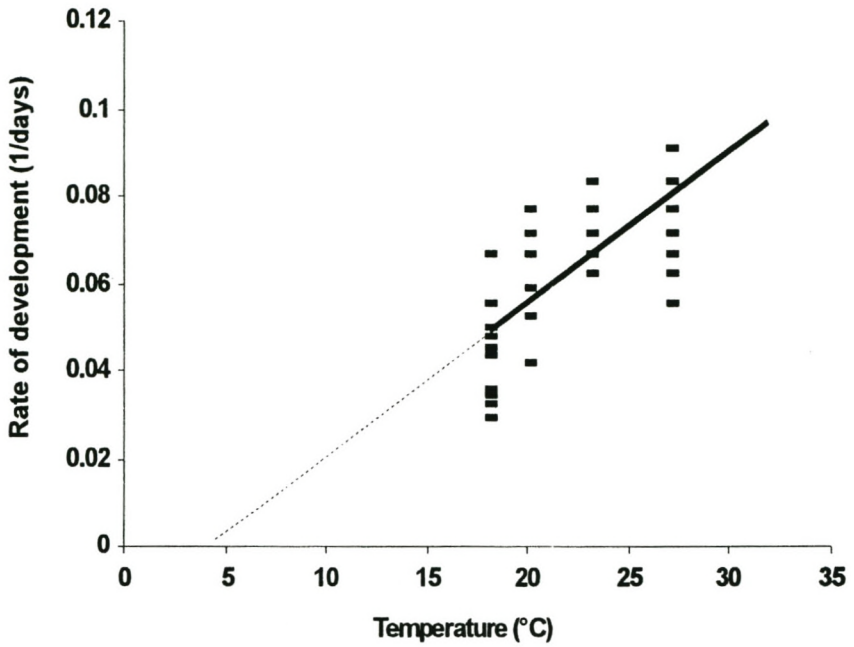

Fig. 3. 2. Regression of rate of development (1/days) on temperature of subterranean *Eriosoma lanigerum*, $Y = -0.01487 + 0.003435(X)$, $r^2 = 0.891067$.

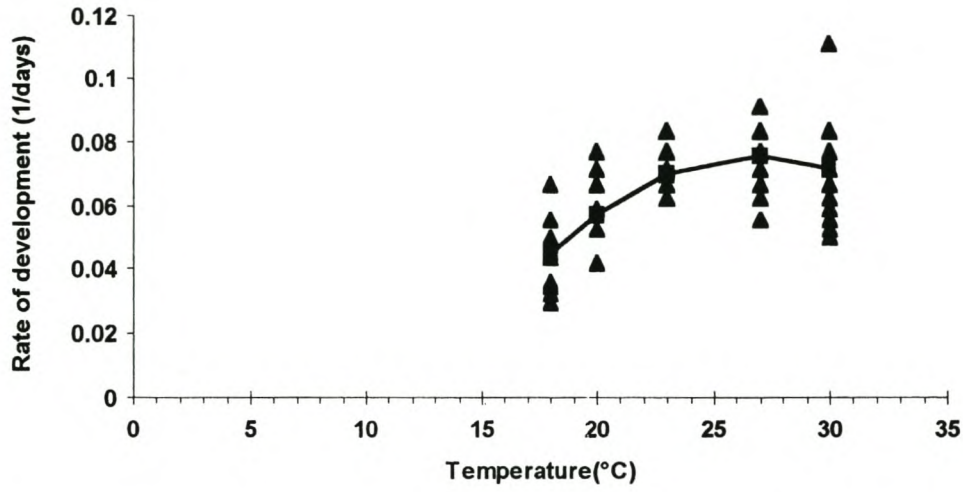

Fig. 3.3. Quadratic regression of developmental rate (1/days) on temperature for subterranean *Eriosoma lanigerum*, $Y=-0.213+0.0215(X)-0.0004(X^2)$; $r^2=0.560$.

3.3 DISCUSSION

The net replacement rate was greater than one and the intrinsic rate was greater than zero at all temperatures except 30 °C, indicating positive population growth.

There is a range of temperatures over which the developmental rate of aphids increases linearly (Dixon 1987). For subterranean *E. lanigerum* this range was between 18 and just below 27 °C (Fig. 3.3).

As temperature increased so the average number of crawlers produced per adult or net replacement rate (R_o) decreased steadily (Fig. 3.4). However, there was little effect on generation time (T) and at 30 °C it did not decrease rapidly (Fig. 3.4). At 30 °C edaphic *E. lanigerum* could survive and complete

its life cycle, but at zero population growth (R_o = 1; Table 3.1). This was due to the low fecundity (Fig. 3.1). Therefore, constant high temperatures were unsuitable for subterranean *E. lanigerum*. Soil temperatures, estimated using the methods described by McWhorter & Brooks (1965), indicated that subterranean populations of *E. lanigerum* would very seldom be subjected to temperatures below 4.32 °C (lower threshold) or above 26.80 °C (upper threshold for optimum development). When this did occur, it was only for a short time. Therefore, under ground temperatures would be favourable for development and reproduction throughout the year.

The highest fecundity was at 18 °C (R_o = 75.34), but the highest intrinsic rate of increase was at 23 °C (r_m.= 0.177). This was probably due to the high rate of production of progeny in early adult life at 23 °C (Fig. 3.1) rather than the total number of nymphs born in the entire lifetime (Dixon 1987). Therefore, it appeared as if the most suitable temperature for subterranean *E. lanigerum* was at about 23 °C.

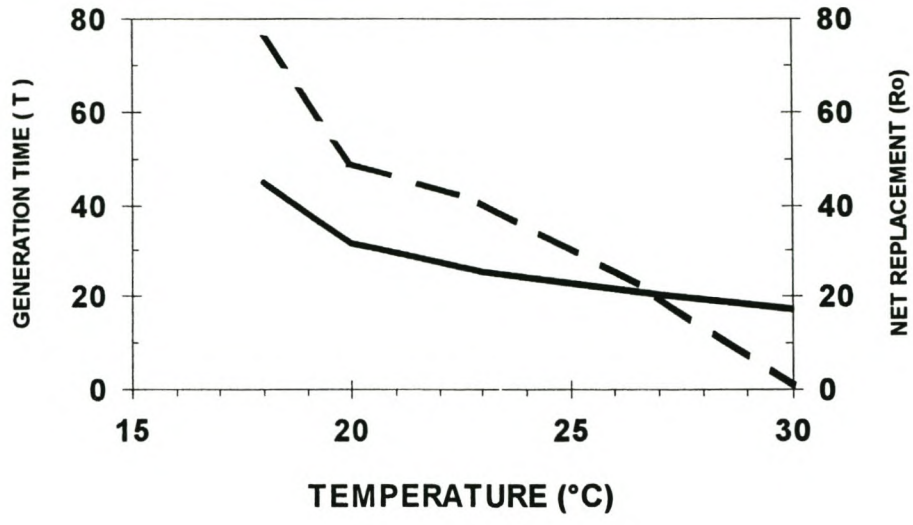

Fig. 3.4. Generation time (solid line) and net replacement rate (broken line) plotted on temperature.

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CHAPTER 4

DEVELOPMENT OF A SAMPLING SYSTEM FOR MONITORING POPULATION LEVELS OF SUBTERRANEAN *ERIOSOMA LANIGERUM* ON APPLE TREES

During spring crawlers from subterranean populations of *Eriosoma lanigerum* (Hausmann) migrate up the trunks of apple trees and settle in leaf axils and pruning wounds where they form colonies. However, there is apparently no correlation between aboveground and underground populations (Brown & Schmitt 1990), indicating that sampling aboveground populations yields no information on the abundance of the *E. lanigerum* on the roots. Therefore, surveys for the latter would have to be done using more difficult (and not yet developed) soil sampling methods (Brown & Schmitt 1990). The object of the present study was to develop such a system.

Other soil pests, such as nematodes, are sampled using augers (Duncan *et al.* 1996, Koppenhofer *et al.* 1998, Neher & Campbell 1996). Therefore, the sampling system used in the present study was also based on the use of augers. In addition, because *E. lanigerum* infests roots, the amount of root material in the samples will affect the number of aphids present. Thus the effects of root material in soil samples was also

investigated. Coker (1959) and Atkinson (1976) showed that there was a concentration of roots in an area of 0.3 to 1.0 m^2 with the trunk at the centre, or in an area with a radius of 0.31 to 0.56 m around the trunk. Therefore, the distance from the trunk at which a sample is taken may affect the number of aphids in the sample. The purpose of the present study was to determine short term changes in population levels of subterranean *E. lanigerum*, as opposed to sampling for making decisions on the necessity for applying control measures.

4.1 MATERIALS & METHODS

4.1.1. Augers used. The standard augers were made from metal piping with an internal diameter of 5.0 cm. They had an external collar 15 cm from the tip which prevented penetration deeper than 15 cm. Therefore, the standard soil sample was 5 cm in diameter and was taken to a depth of 15 cm. When the soil was moist these samples could easily be taken with a light hand auger. However, in hard, dry soil a mechanical auger was sometimes used. This was a Stihl BT 106 earth auger fitted with an auger of the type described above.

4.1.2. Treatment of samples. All samples were placed into plastic packets and transported to the laboratory in cool bags. *E. lanigerum* was washed out of the soil into a sieve with a mesh size of 200μ, as previous measurements of 260 *E. lanigerum* crawlers indicated that they would not pass through this mesh size. All developmental stages were counted. They were classified as alive, dead or dry. Those that were dead were still in good condition and were assumed to have been killed during sampling, transporting or washing. Therefore, alive and dead aphids were included in the data analyses. The dry aphids were assumed to have been dead prior to sampling and were not included in the analyses.

4.1.3. Effects of distance from the trunk and type of auger. These aspects were investigated in a series of experiments. The distances from the trunks which were investigated could not be compared in one trial because the disturbance of the soil and destruction of roots during sampling at one point may have influenced the number of aphids occurring in adjacent samples. Distances of 20, 30, 40 and 60 cm from the trunk were tested. In experiment 1 two distances from the trunk, 30 and 60 cm, were compared using the mechanical auger. These two distances were tested at each of 24

trees and the data were analysed as a randomised block design with trees as blocks after a log (X+1) transformation had been applied to stabilise the variances. In experiment 2, two distances from the trunk (20 and 40 cm) and the two augers were compared in a factorial design with trees as blocks. The main effects were distance from the trunks and auger. Thirteen trees were used. The data were analysed in a factorial design after a log (X+1) transformation had been applied to stabilize the variances. In experiment 3 the hand auger was compared with the mechnical auger. Samples were taken 30 cm from the trunks at each of 25 trees using the two augers. The data were analysed as described in experiment 1.

4.1.4. Influence of root material. Soil samples were taken at regular intervals throughout the year 30 cm from the trunks of 12 to 13 evenly spaced apple trees in each of the four 2 ha blocks described in the Introduction. Sampling was not carried out at the same tree on successive dates as sampling was destructive in that root material was removed by the auger. Approximately six months elapsed before a subsequent sample was taken from a given tree. Sampling continued for two years and three months. When the soil samples were washed they were classified as follows according to the root material they contained:

0 = soil sample containing no roots or galls;

1 = sample containing roots with no galls;

2 = < 25% of the roots in the sample galled;

3 = > 25% < 75% of the roots in the sample galled and

4 = > 75% of the roots in the sample galled.

Spearman's rank correlation was used to determine whether or not there was a correlation between the amount of root material and galls in the samples and the number of aphids.

The influence of root material in the samples on dispersion statistics was investigated using Taylor's power law (Taylor 1984) by employing dummy variables (Gujarati 1970) to examine the effects of the five categories of soil samples on the regression constants in Taylor's power law. In the full model the five categories of soil samples were assumed to have separate intercepts and slopes, while in the reduced model all five categories of soil samples were assumed to have a common intercept and a common slope.

4.2 RESULTS & DISCUSSION

4.2.1 Effects of distance from the trunk and type of auger. In experiment

1 the apparently large difference in aphid numbers between samples taken 30 cm and 60 cm from the trunk (Table 4.1) borded on significance ($P = 0.06$, Table 4.2). In experiment 2 many more aphids were recorded in samples taken 20 cm from the trunks than in those taken 40 cm from the trunk (Table 4.1). These differences were highly significant ($P < 0.001$, Table 4.2).

Differences in aphid numbers between samples taken with the hand and mechanical auger were not large (Experiment 2 and 3, Table 4.1) and were not significant ($P = 0.88$ and 0.70 in experiment 2 and 3 respectively, Table 4.2). From these results it is clear that the distance from the trunk at which samples were taken influenced the number of aphids in the samples. It was decided to standardise the distance from the trunk at which samples are taken at 30 cm, because Atkinson (1976) showed that there was a concentration of roots between about 30 and 55 cm from the trunk. The type of auger used for taking samples did not affect the number of aphids recorded. Therefore, either the mechanical or hand auger can be used for future sampling.

4.2.2. Influence of root material. There was a strong positive correlation between the soil samples categorised according to the root material found in

them and the number of *E. lanigerum* in the samples (R_s = 0.94, d.f.= 40, P < 0.001). Therefore, as the degree of galling of roots in the soil samples increased so the number of aphids in the samples also increased.

The regression of log variance on log average fitted the data well (R^2 = 0.94; $F_{1;38}$ = 595.28; P < 0.001) indicating good fit by Taylor's power law (Fig. 4.1). The full model, with separate intercepts and slopes for the five categories of soil samples, could be reduced to one with a common intercept and slope ($F_{8;30}$ = 2.01; P = 0.08), indicating that the dispersion statistics were not influenced by the root material in the soil samples. The slope of 1.82 suggested an aggregated dispersion pattern and the common intercept (a = 0.773) suggested that the background variance (Taylor 1984) was constant regardless of the root material in the samples. From Fig. 4.1 it is also clear that the average number of aphids in soil samples containing highly galled roots was greater than in those containing no roots or few galls, confirming the conclusions drawn from the Spearman's rank correlation. Therefore, future sampling will be conducted using either the hand or mechanical auger, depending on soil texture. All samples will be taken 30 cm from the trunks of trees, as distance affects the number of aphids in the soil. However, the presence or absence of roots and/or galls in the samples will unavoidably

lead to a high degree of variability between samples. This explains why the apparently large differences in aphid numbers between samples taken 30 and 60 cm from the trunks (Experiment 1, Table 4.1) were not clearly significant (P = 0.06, Table 4.2).

Table 4.1. Average number of aphids in samples taken with different augers and at different distances from the trunk. The ticks (✓) indicate which auger or distance from the trunk was used.

	Auger		Distance			
Experiment	**Hand**	**Mechanical**	**20**	**30**	**40**	**60**
1	-	✓	-	19.21	-	4.17
2	20.38	12.35	30.81	-	1.92	-
3	28.76	6.64	-	✓	-	-

Table 4.2. Analysis of variance results in 3 experiments comparing counts of *E. lanigerum* taken using different augers and at different distances from the trees. Data were transformed using Log (X+1) prior to analysis.

Experiment	Source	df	Ms	F	P
1	Distance (30 vs 60)	1	0.4287	3.806	0.06
	Tree (Block)	23	0.6588	5.848	<0.001
	Error	23	0.1126		
2	Distance (20 vs 40)	1	5.8709	16.175	<0.001
	Auger	1	0.0077	0.021	0.88
	Distance * Auger	1	0.0015	0.004	0.95
	Tree	12	0.4761	1.312	0.25
	Error	36	0.3629		
3	Auger	1	0.0271	0.148	0.70
	Tree (Block)	24	0.6800	3.701	0.001
	Error	24	0.1837		

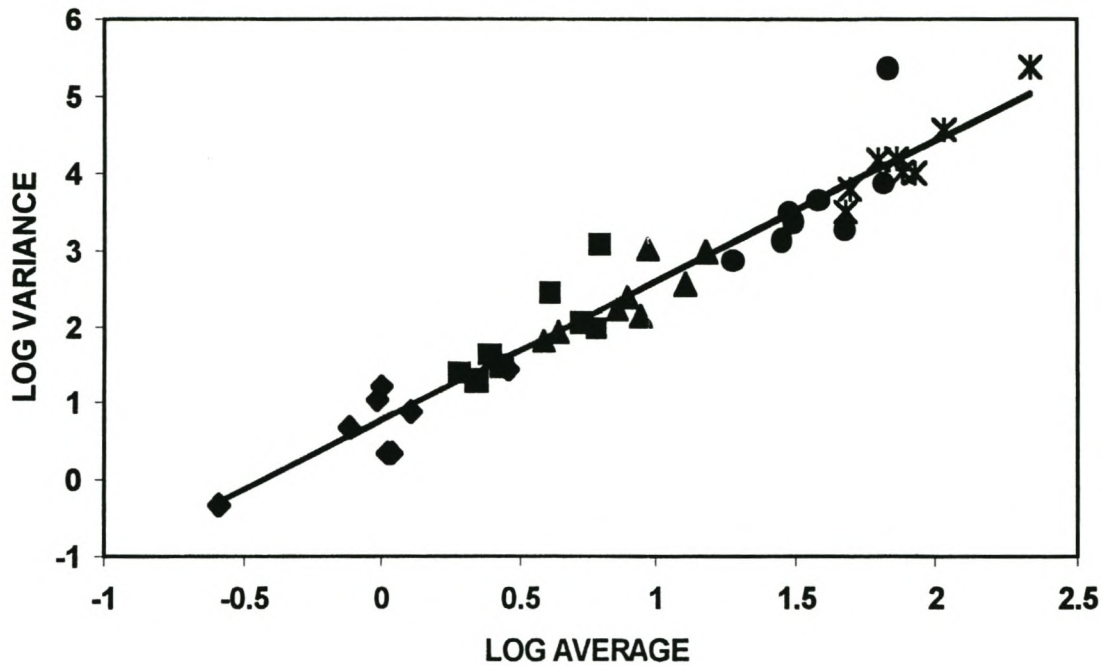

Fig. 4.1. Regression of log variance on log average for soil samples, $Y = 0.773 + 1.823(X)$, $r^2 = 0.94$. Diamonds = soil samples containing no roots; squares = roots with no galls; triangles = roots with < 25% galls; circles = roots with > 25% < 75% galls and crosses = roots with > 75% galls.

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CHAPTER 5

BIOLOGY OF SUBTERRANEAN *ERIOSOMA LANIGERUM*

There is little information on the underground activities of *Eriosoma lanigerum* (Hausmann), but crawler movement between the subterranean and aerial parts of the tree has been reported by some authors. In Palestine, aerial and root colonies were observed to develop independently, with little movement between them (Bodenheimer 1947). However, in New South Wales (Australia) young aphids wandered in an aimless manner with random movement between roots and aerial parts of the apple tree (Froggatt cited by Hoyt & Madson 1960). There are reports also of a relationship between seasons and crawler movement. For instance, in America there was an upward movement in the early summer and movement from the limbs to the roots in the fall (Hoyt & Madson 1960), while Lohrenz (1911) believed that aphids moved to the roots when it was hot. However, in England, overwintering occurred on the upper parts of the tree and a definite migration upwards from the roots was not recorded (Greenslade 1936). In contrast, in South Africa, large numbers of crawlers migrated up the trunks in spring and infested leaf axils (Pringle 1998). Thus, there are conflicting reports regarding the interaction between aerial and subterranean populations of *E. lanigerum*.

The object of the present study was therefore to obtain information on the biology of subterranean populations of *E. lanigerum* in the Western Cape Province of South Africa.

5.1 MATERIAL AND METHODS

5.1.1 Population studies. The orchards described in the Introduction were used.

Soil samples were taken at regular intervals from 12 to 13 trees in each of the four blocks. The soil augers described in Chapter 4 were used to take standard soil core samples 15 cm deep and 30 cm from the trunks of the trees. The samples were treated in the same way as described in Chapter 4. The *E. lanigerum* individuals collected in the sieves were counted and the different developmental stages identified.

Data on the upward migration of *E. lanigerum* were obtained from Heunis (thesis in preparation). The data were used to determine whether or not there was a relationship between the underground population and the upward movement of *E. lanigerum* crawlers. Upward migration was measured by Heunis (thesis in preparation) using masking tape bands placed low down around the trunks of five trees per block in each of four blocks. A five centimetre length of the masking tape was painted with Plantex®, which

trapped the crawlers moving up the trees. For more details see Heunis (thesis in preparation).

5.1.2 Age structure. Sixty trees were selected in block two on Molteno Trust and in block one on Oak Valley. Five of these 60 trees in each block were randomly chosen and infested roots were collected and transported to the laboratory in a cool bag at weekly intervals. If some of the trees were not infested, a tree next to it in a parallel row that was not included in the original 60 trees was sampled. Aphids were washed off the roots into a sieve as described above. One hundred *E. lanigerum* were removed at random and the percentages of each developmental stage represented were determined.

5.1.3 Nitrogen determination. Samples of roots were taken while collecting soil samples in all the blocks. The roots were transported to the laboratory where they were washed and cut into pieces of approximately 3 mm. They were kept frozen until the percentage nitrogen in them could be determined. This was done using a Leco Nitrogen Analyser, in which there was total combustion and the amount of nitrogen was determined by thermal conductivity.

5.2 RESULTS

5.2.1. Population studies. There was a similar pattern in the population fluctuations of subterranean *E. lanigerum* in both blocks on Molteno Trust and Oak Valley Estate (Figs. 5.1A, 5.2A, 5.3A and 5.4A). There usually appeared to be two population peaks a year, one during early summer (November, December) and one during autumn from February to April. At both sites (Molteno and Oak Valley) more subterranean aphids were recorded during the 1997/98 season than during the other two seasons (Figs. 5.1A, 5.2A, 5.3A and 5.4A).

During the winter (June, July, August) of 1996 subterranean *E. lanigerum* populations and the upward movement of crawlers were low (Figs. 5.1A, B; 5.2A, B; 5.3A, B and 5.4A, B). However, during the winter of 1997 there was not a marked decline in aphid numbers (Fig. 5.1A, 5.2A, 5.3A and 5.4A).

Upward movement of crawlers occurred mainly during the first half of the summer, indicating that only the first peak in subterranean aphid numbers produced upward migration of crawlers (Figs. 5.1B, 5.2B, 5.3B and 5.4B).

5.2.2 Age structure. All developmental stages of *E. lanigerum* were observed on the roots throughout the year (Fig. 5.5). There was no clear

pattern in the percentages of subterranean *E. lanigerum* in the 2nd, 3rd, 4th instars and adult stage of each generation. However, the percent of first instar nymphs increased during late spring and early summer (October, November, December) and decreased during winter (June, July, August).

5.2.3 Nitrogen determination. The percent nitrogen in roots was very variable, but tended to be higher during early summer (November, December) than in autumn (February, March) (Figs 5.1C, 5.2C, 5.3C and 5.4C). However, during 1997/98 in block one on Molteno the nitrogen level in November and December was slightly lower than in February and March (Fig. 5.1C), and during 1995/96 in block two on Oak Valley the nitrogen level in February was higher than November and December (Fig. 5.4C).

5.3 DISCUSSION

There were usually two peaks in numbers of subterranean woolly apple aphids, one during early summer and one during autumn. These coincided with peak levels of movement of amino acids in the roots (Titus & Seong-Mo Kang 1986). During the first peak, numbers of first instar nymphs also reached a peak and at this time crawlers moved up the trunk to infest leaf axils, as was also found by (Pringle 1998). However, Bodenheimer (1947) indicated that aerial and root colonies developed independently. In addition a

definite migration upward from the roots was not recorded by Greenslade (1936). The second peak in numbers of edaphic *E. lanigerum* coincided with a slight increase in the percent first instar nymphs (Fig 5.5). Furthermore, high numbers of aphids migrating down the trunks were not recorded (Heunis, Ph.D. thesis in preparation), nor were aphids which had dropped from the aerial parts of the trees observed (personal observation). Therefore, it appeared as if the second peak in subterranean *E. lanigerum* numbers originated from those already on the roots.

During the winter (June, July, August) of 1997 subterranean *E. lanigerum* populations were higher in comparison with the 1996 and 1998 winter seasons. Lower rainfall was recorded during the 1997 winter period compared to the other two years (Fig. 5.6). It would, therefore, seem that high rainfall could have a suppressing effect on subterranean *E. lanigerum* population levels.

The early summer increase in subterranean *E. lanigerum* populations also coincided with peak levels of nitrogen in the roots. It is not clear, however, if there was a causal relationship between underground aphid numbers and nitrogen levels in the roots.

It was not possible to determine the number of generations per year directly from the data. However, the daily maximum and minimum soil

temperatures were determined from air temperatures as described by McWhorter & Brooks (1965). Using these estimates, the lower threshold temperature of 4.32°C and the estimated 291.12 °D required for development (see Chapter 3), the estimated number of generations per year was approximately 18.

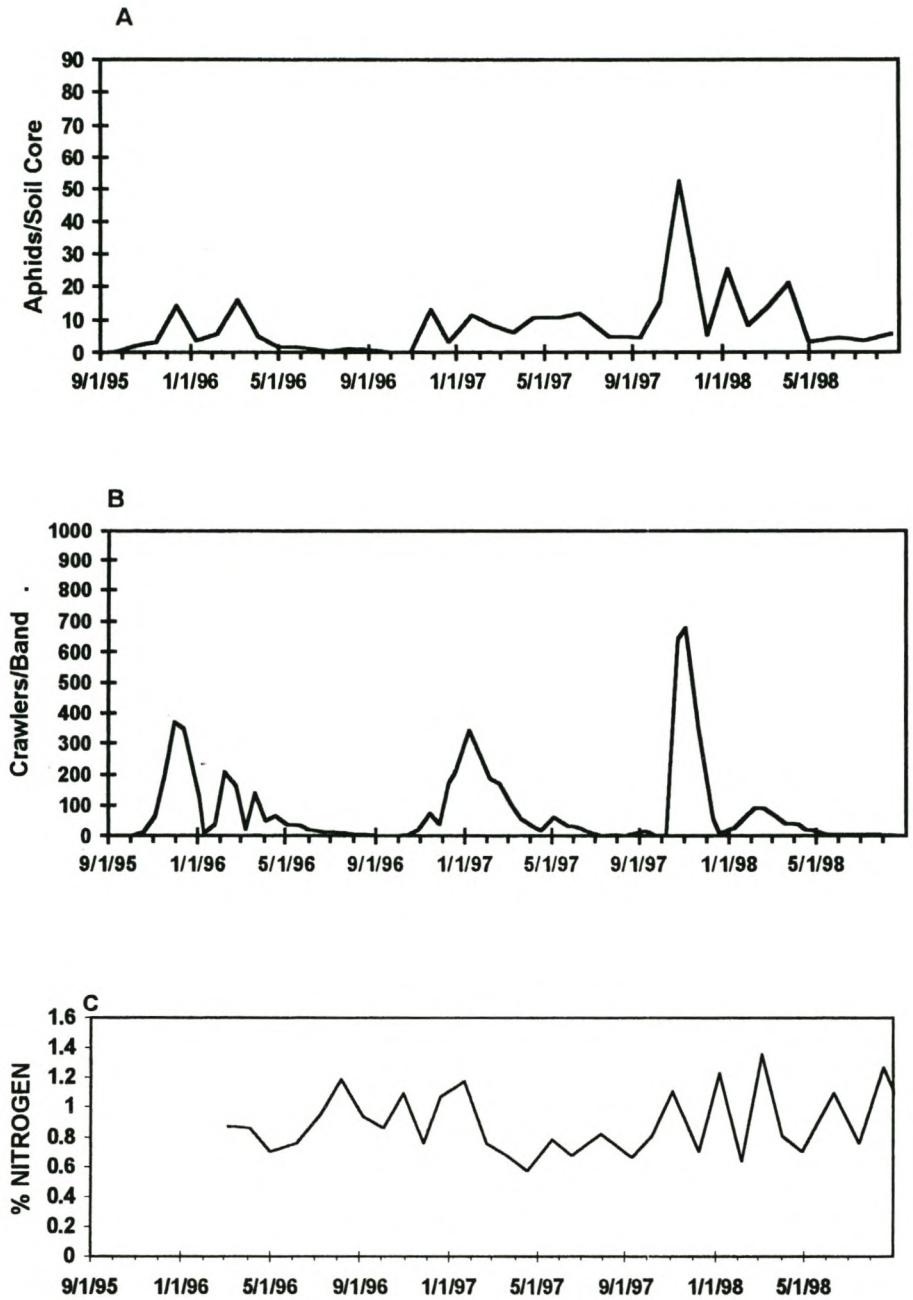

Fig. 5.1. Number of *Eriosoma lanigerum* per soil core sample (A); number of crawlers per 5 cm masking tape band (B); average percent nitrogen in roots (C) of apple trees in block 1 on Molteno (date format MONTH/DAY/YEAR).

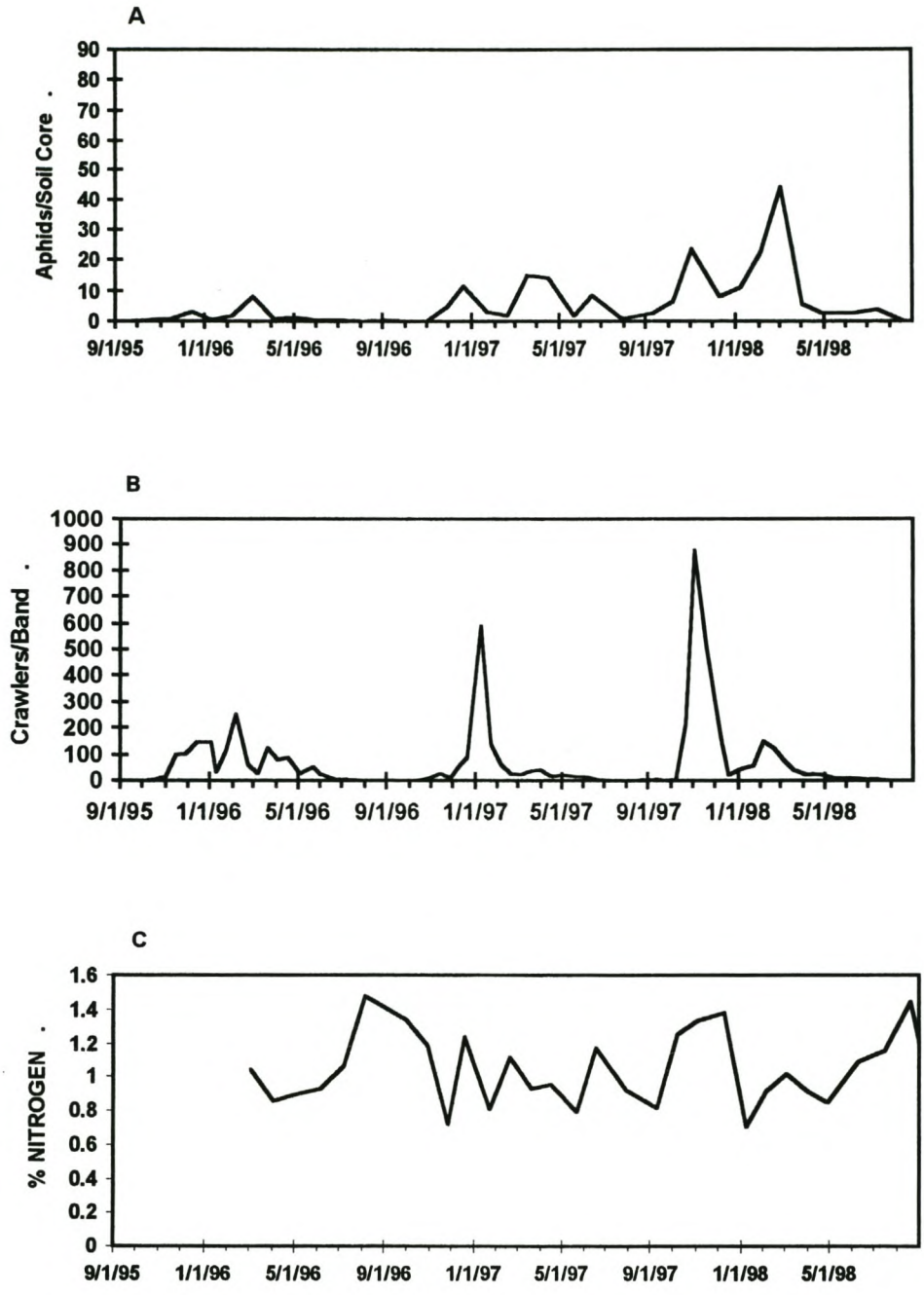

Fig. 5.2. Number of *Eriosoma lanigerum* per soil core sample (A);number of crawlers per 5 cm masking tape band (B); average percent nitrogen in roots (C) of apple trees in block 3 on Molteno (date format MONTH/DAY/YEAR).

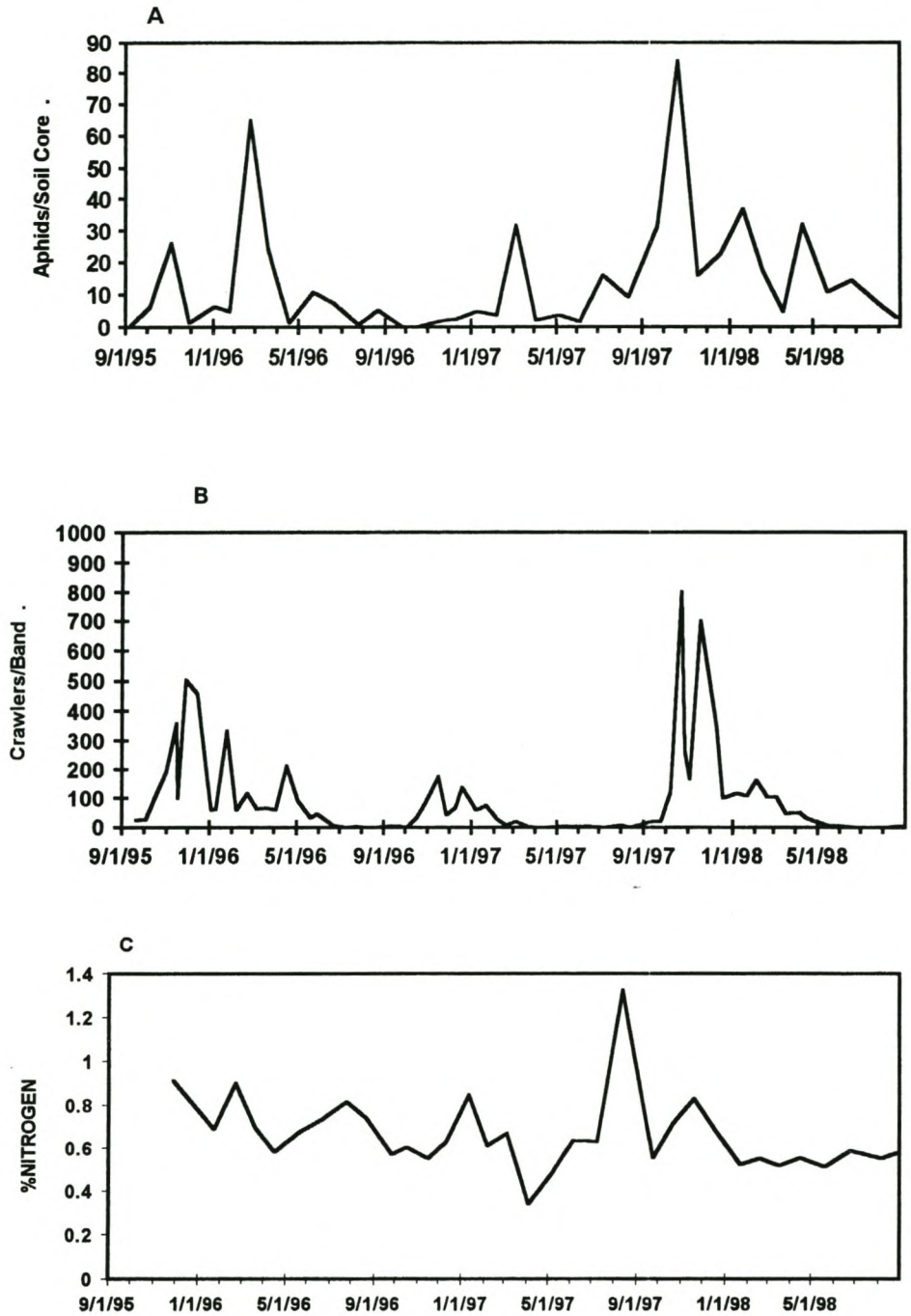

Fig. 5.3. Number of *Eriosoma lanigerum* per soil core sample (A); number of crawlers per 5 cm masking tape band (B); average percent nitrogen in roots (C) of apple trees in block 1 on Oak Valley (date format MONTH/DAY/YEAR).

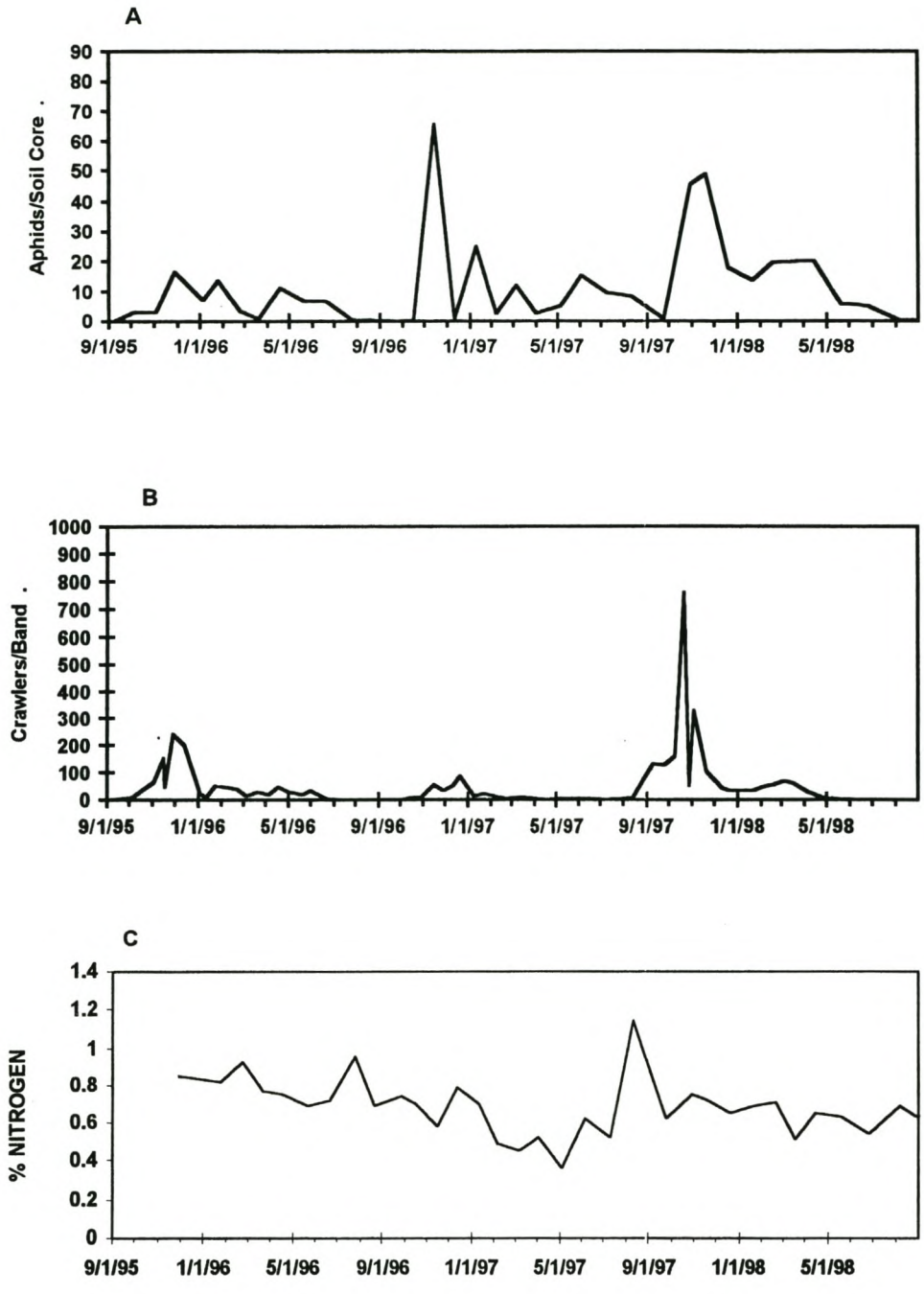

Fig. 5.4. Number of *Eriosoma lanigerum* per soil core sample (A); number of crawlers per 5cm masking tape band (B); average percent nitrogen in roots (C) of apple trees in block 2 on Oak Valley (date format MONTH/DAY/YEAR).

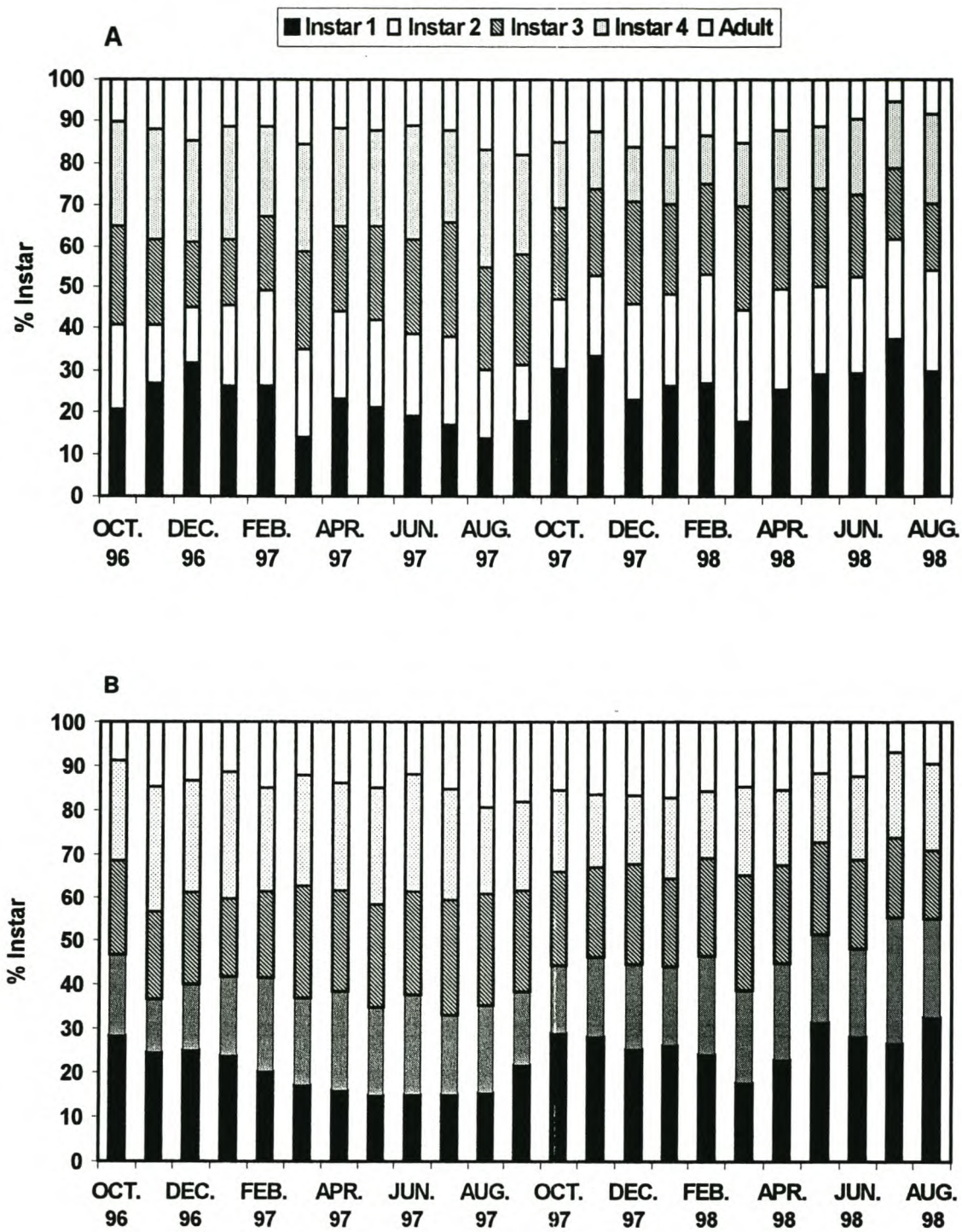

Fig. 5.5. Percent subterranean *E. lanigerum* in each instar. A, Molteno; B, Oak Valley.

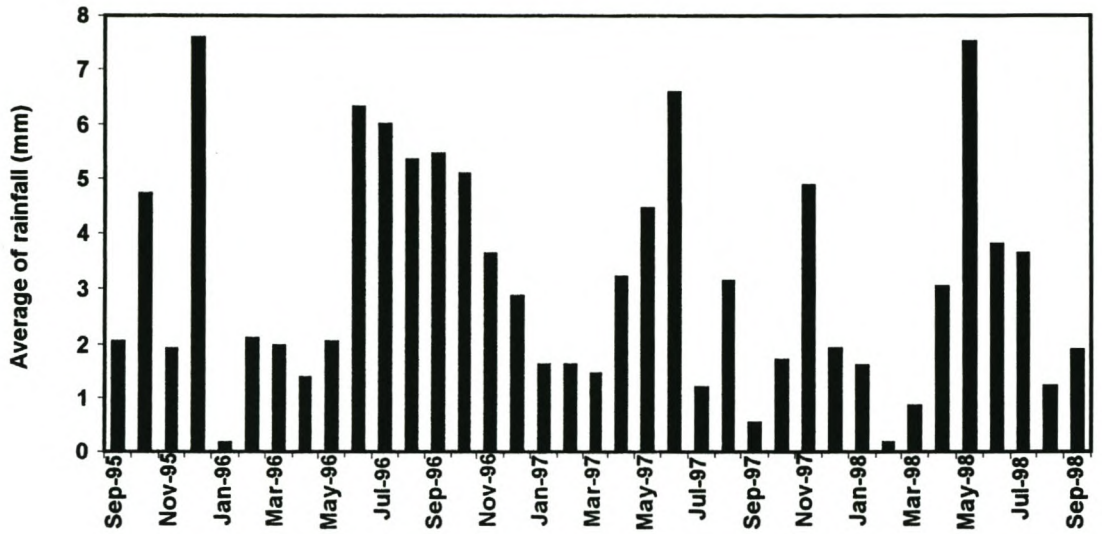

Fig. 5.6. Average monthly rainfall recorded in Elgin.

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CHAPTER 6

INTERACTION BETWEEN *ERIOSOMA LANIGERUM* AND THE ROOTS OF APPLE TREES

Two extrinsic factors, food quality and temperature, can affect the reproductive potential of aphids (Dixon, 1987). Of the nutritional factors that determine food quality, nitrogen is considered to be one of the most important for the growth and development of apple trees (Titus & Seong-Mo Kang 1986). However, the development of aphids is also closely linked to the nitrogen content of the host plants (Klingauf 1987). In addition, galls on apple roots resulting from feeding by subterranean *E. lanigerum* contain higher nitrogen levels than adjacent, healthy root tissue (Brown *et al.* 1991). The present study was conducted to determine: (1) whether or not there was a relationship between nitrogen content of the roots of apple trees and breeding cycles of woolly apple aphid and (2) to compare the nitrogen levels in normal roots with those in galls.

6.1 MATERIAL & METHOD

6.1.1. Breeding cycles. The sites used were those described in the Introduction. Aphids washed from soil samples taken from the four blocks on

the two farms described in Chapter 5 were used. Ten aphids from each of the five developmental stages (four nymphal instars and adults) were randomly selected and dissected to reveal the ovarioles. The embryos were counted using phase contrast and dark field microscopy. Samples from both sites were taken at approximately monthly intervals but not on the same date. The data were analysed using a factorial analysis of variance with instar and date as main effects. Because samples from both farms were not taken on the same date, data from the two farms were analysed separately. Differences between groups of means were tested using orthognal contrasts (Snedecor & Cochran 1980).

6.1.2. Nitrogen. Samples of roots were collected at regular intervals throughout the year from the two sites as described in Chapter 5. The root samples were divided into three categories: healthy roots, normal root sections taken adjacent to a gall, and galled roots. Roots were transferred to the laboratory, where the soil was washed off. The samples were cut into small pieces of approximately 3 mm. They were kept frozen until they were analysed for nitrogen content as described in Chapter 5. The data were analysed in a factorial design with the blocks of apple trees and the root categories as main effects. Dates were used as replicates. Differences

between groups of means were tested using orthognal contrasts (Snedecor & Cochran 1980).

A second experiment was conducted to verify the results of the experiment described above. Ten trees in block 2 on Molteno and ten trees in block 2 on Oak Valley were selected. The trees selected were not surrounded by a solid mat of root galls around the trunk. This was done to ensure that roots classed as "normal" were not actually roots attached to galls close to the trunk. From each tree, the three categories of root samples were collected and treated as described in experiment one. The data were analysed in a factorial design with farms and root categories as main effects. Sampling was done during December 1998.

6.2 RESULTS

6.2.1. Breeding cycles. In both orchards (Molteno and Oak Valley), embryos were present in all developmental stages (Figs. 6.1A and B). There were interactions between instar and date on both farms, indicating that these differences between instars were not the same on all dates. However, the F-values for these interactions were small relative to the F-value for differences between dates and especially for differences between instars (Tables 6.1 and 6.2). Therefore, these interaction would have only a small effect on

differences between instars. The extremely high F-values for instar were due to noticeably more embryos being present in 4th instar and adult aphids than in the other instars (Fig. 6.1A and B). These differences for Molteno ($F_{1:1809}$ = 1441.77; P<<0.001) and for Oak Valley ($F_{1:1739}$ = 1637.07; P<<0.001) were highly significant.

The maximum number of embryos in 4th instar and adult aphids occurred during spring (September to October) (Fig. 6.1A and B). However, there was a higher total number of embryos in all instars during winter (June, July, August) (Fig. 6.2A and B). This was due largely to higher numbers of embryos in the first three instars during this time of the year.

6.2.2. Nitrogen. The percentage of nitrogen in the roots was also higher during spring and early summer than in late summer and early autumn (Fig. 6.2A and B). There were interactions between blocks and root categories ($F_{6:264}$ = 2.429; P = 0.026). Therefore, data from the two farms were analysed separately. There were differences in nitrogen content between the three root categories on both Molteno Trust and Oak Valley (Fig. 6.3). These differences were highly significant (Table 6.3 and 6.4). Nitrogen levels in normal roots adjacent to nodules and in healthy roots were higher than those in root nodules on both farms (Fig. 6.3). These differences for Molteno (t_{132} = 6.45;

P<0.001) and Oak Valley (t_{132} = 4.56; P<0.001) were significant. Nitrogen levels in roots differed between blocks and seasons on Molteno Trust (Table 6.3) but not on Oak Valley (Table 6.4).

In the second experiment there were differences in nitrogen levels between farms (Fig. 6.4). These differences were significant (Table 6.5), with higher nitrogen levels on Molteno than on Oak Valley (Fig. 6.4). There were also differences between categories (Fig. 6.4; Table 6.5). Nitrogen levels in ungalled roots and in roots taken adjacent to galls appeared to be similar but differed from those in galls (Fig. 6.4). This difference was significant (t_{54} = 3.61; P<0.001).

6.3 DISCUSSION

Spring generation aphids commonly have more ovarioles than generations during the remainder of the year (Welling *et al.* 1980). In the present study ovarioles were not counted, but the number of embryos in 4th instar and adult aphids was higher during spring than during the remainder of the year. This co-incided with peak levels of nitrogen in the roots of apple trees. However, it appeared as if nitrogen level was not the only factor which affected the numbers of embryos, because nitrogen levels on Molteno were consistently higher than on Oak Valley (Figs. 6.3 and 6.4), while there

appeared to be more embryos in aphids from Oak Valley than in those from Molteno (compare Figs. 6.1A with 6.1B and Fig. 6.2A with 6.2B). The rootstock on Oak Valley was seedling (see Introduction) which is known to be more susceptible than M 793 (Giliomee *et al.* 1968), the rootstock on Molteno.

Therefore, fluctuations in nitrogen levels in the roots may help explain fluctuations in the seasonal production of embryos, but the overall fecundity of subterranean *E. lanigerum* populations appears to be determined by other factors.

Higher nitrogen levels were recorded in ungalled roots and in roots adjacent to galls than in root galls. This contradicts the findings of Brown *et al.* (1991) who reported higher nitrogen levels in root galls. However, Brown *et al.* (1991) reported that the aphid induced galls were 0.3 to 1.8 mm long, while the galls analysed in the present study were considerably larger, often being taken from a mat of galled roots which had probably formed over a long period of time as the trees were 18 (Oak Valley) to 26 (Molteno) years old (see Introduction). Therefore, it is possible that initially the galls act as a nitrogen sink as described by Brown *et al.* 1991) but as they become older, the disruption of translocation into the galled area results in a decline in nitrogen levels. Nitrogen may then accumulate where ungalled roots attach to the galls, creating a favourable feeding substrate for the aphids.

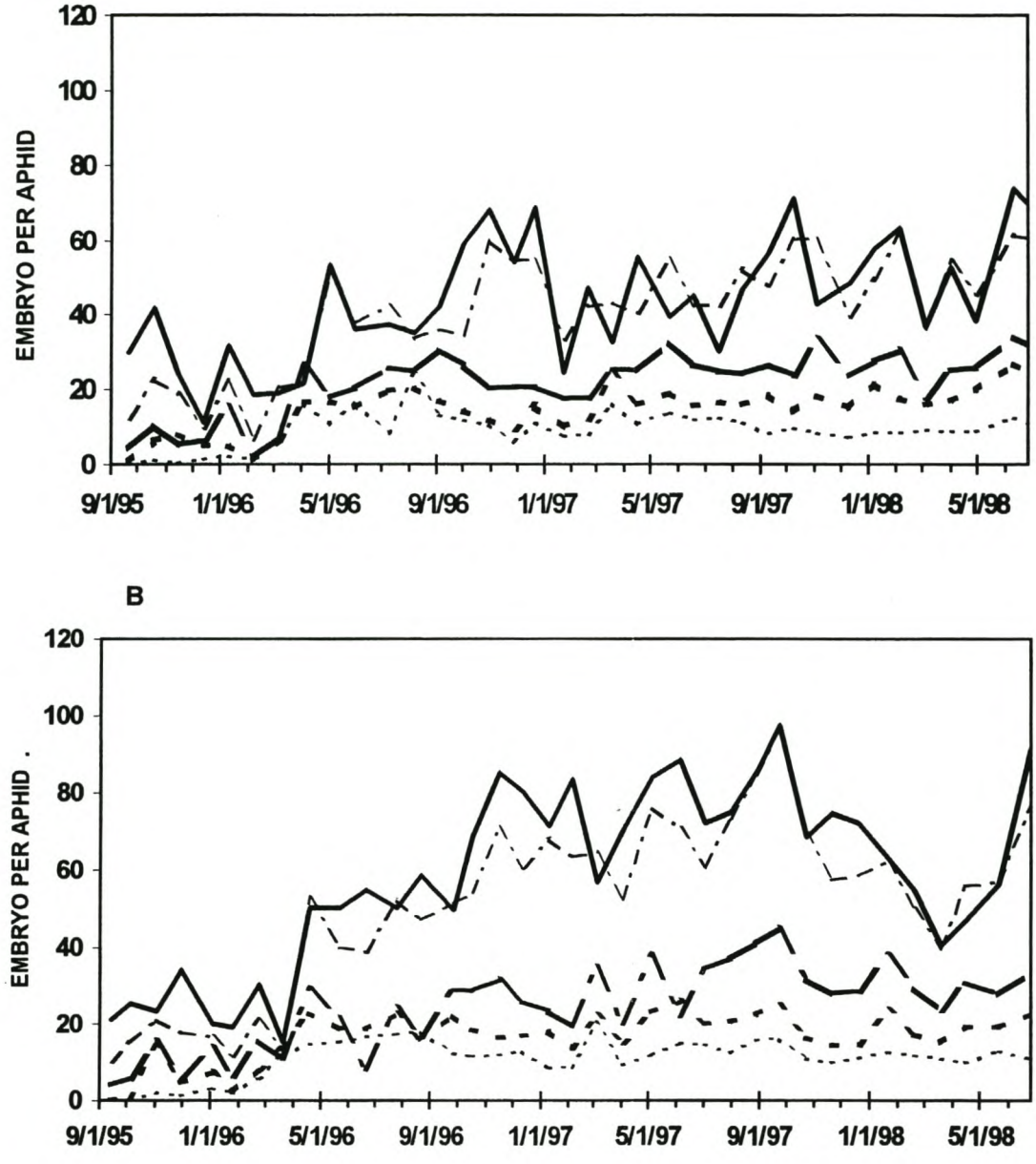

Fig. 6.1. Number of embryos per aphid in the different developmental stages of subterranean woolly apple aphids on A, Molteno; B, Oak Valley. Adult = solid line; 4th instar = thin broken line; 3rd instar = thick broken line; 2nd instar = thick dotted line; 1st instar = thin dotted line.

Table 6.1. Factorial analysis of number of embryos in 5 developmental stages (instars) of subterranean *E. lanigerum* sampled during 12 months (Date) on Molteno.

SOURCE	df	MS	F	P
Date	11	15393.91	7.568	<0.001
Instar	4	87389.83	429.483	<0.001
Date x Instar	44	546.44	2.685	<0.001
Error	1751	203.47		

Table 6.2. Factorial analysis of number of embryos in 5 developmental stages (instar) of subterranean *E. lanigerum* sampled during 12 months (Date) on Oak Valley.

SOURCE	df	MS	F	P
Date	11	4434.7	13.951	<0.001
Instar	4	156636.8	492.772	<0.001
Date x Instar	44	846.6.	2.663	<0.001
Error	1684	317.868		

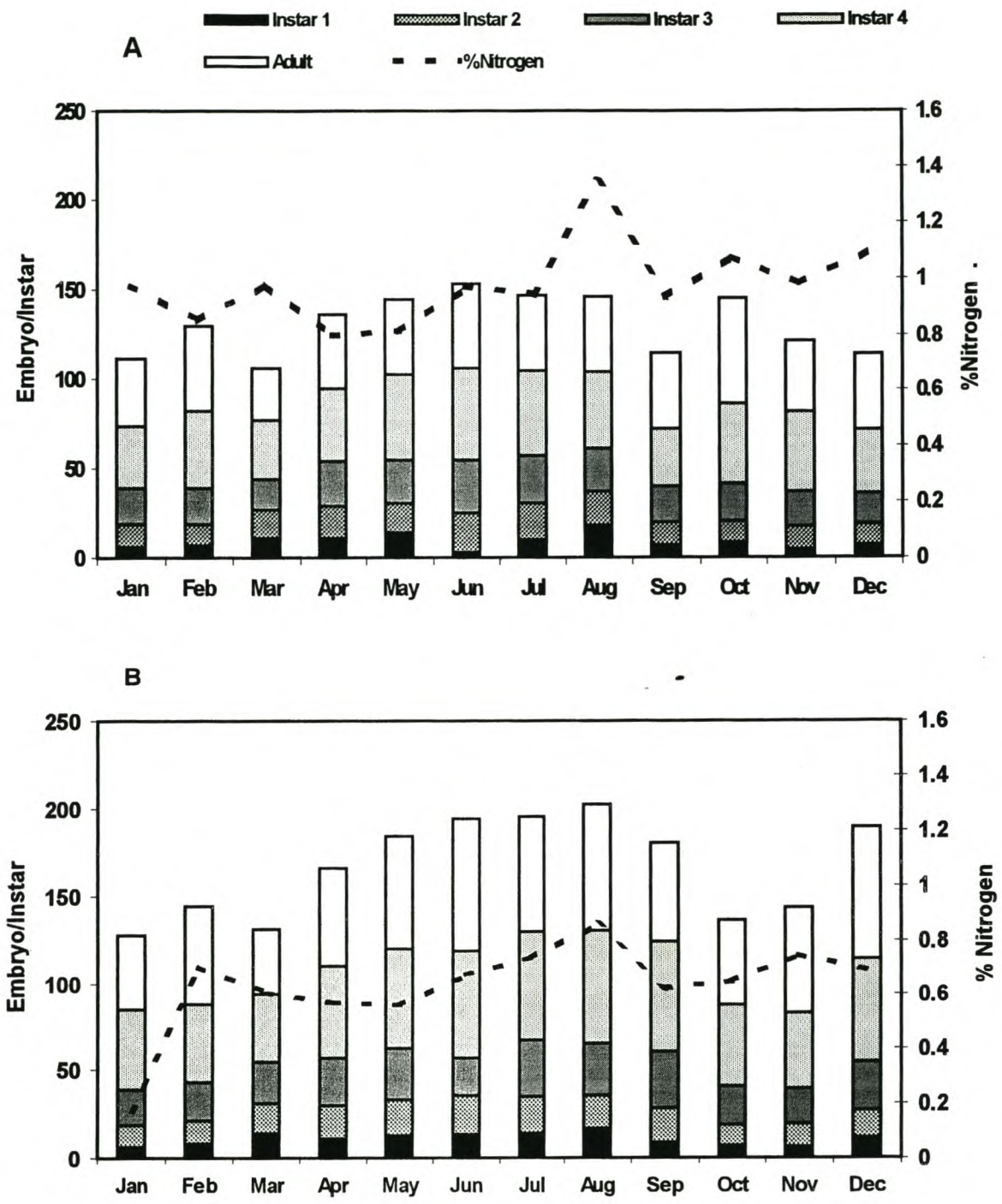

Fig. 6.2. Average number of embryos in each instar and adult subterranean *E. lanigerum* and average percent nitrogen in root of apple trees during different months of the year on, A, Molteno; B, Oak Valley.

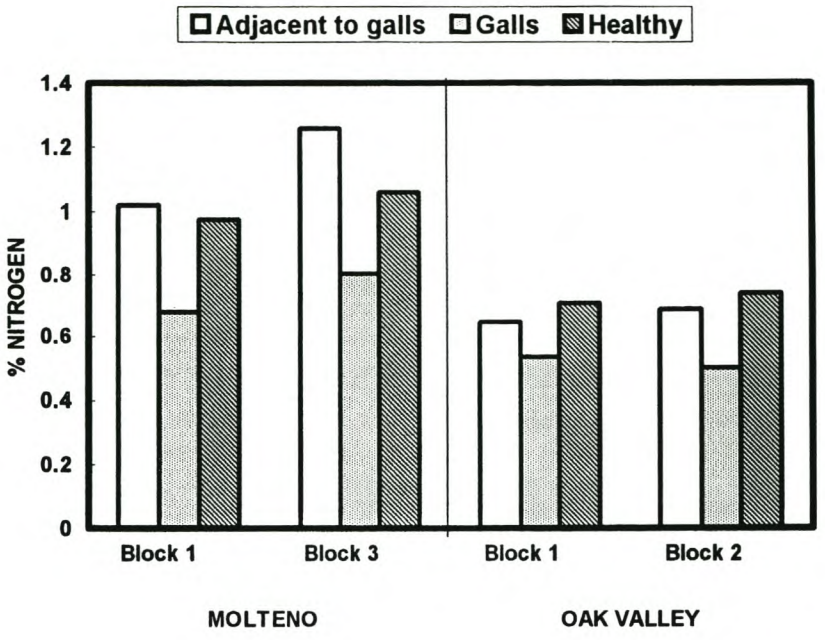

Fig. 6.3. Percent nitrogen in ungalled roots, normal roots adjacent to galls, and in galls.

Table 6.3. Factorial analysis of percent nitrogen in three root categories during two years from two blocks on Molteno.

SOURCE	df	MS	F	P
Block	1	0.811	9.388	0.002
Year	1	0.369	4.269	0.040
Category	2	1.974	22.840	<0.001
Block x Year	1	0.022	0.263	0.608
Block x Category	2	0.076	0.888	0.413
Year x Category	2	0.111	1.286	0.279
Block x Year x Category	2	0.005	0.060	0.941
Error	132	0.086		

Table 6.4. Factorial analysis of percent nitrogen in three root categories from two blocks on Oak Valley.

SOURCE	df	MS	F	p
Block	1	0.005	0.114	0.735
Year	1	0.008	0.171	0.679
Category	2	0.541	11.248	<0.001
Block x Year	1	0.064	1.342	0.248
Block x Category	2	0.021	0.437	0.646
Year x Category	2	0.021	0.452	0.637
Block x Year x Category	2	0.022	0.472	0.624
Error	132	0.048		

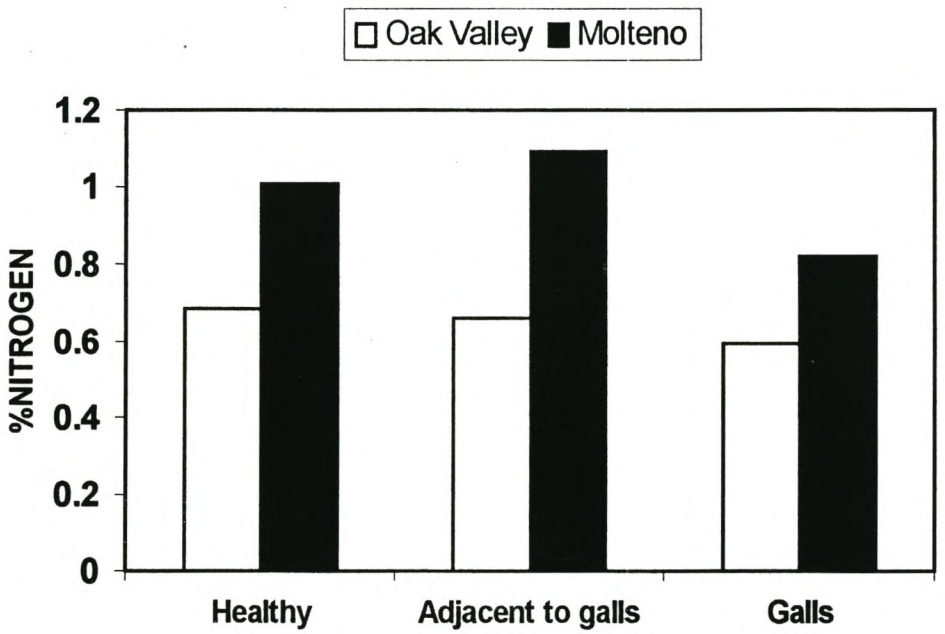

Fig. 6.4. Percent nitrogen in three categories of the root of apple trees.

Table 6.5. Factorial analysis of percent nitrogen in three root categories from Molteno Trust and Oak Valley.

SOURCE	df	Ms	F	P
Farm	1	1.600	64.856	<0.001
Category	2	0.165	6.702	0.002
Farm x Category	2	0.051	2.069	0.136
Error	54	0.246		

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CHAPTER 7

FUNGI ASSOCIATED WITH SUBTERRANEAN *ERIOSOMA LANIGERUM*

Subterranean populations of *Eriosoma lanigerum* (Hausmann), feeding on roots of apple trees, occur in an environment which protects them from the most common biological control agent of aerial populations, the parasitic wasp, *Aphelinus mali* (Haldeman) (Mueller *et al.* 1992). No parasitic or predatory arthropods have been observed attacking the subterranean population (Pringle, personal communication). However, Brown *et al.* (1992) reported on the use of a parasitic nematode for the control of subterranean *E. lanigerum* and Asante (1994) found pathogenic fungi infecting aerial populations of these aphids. There have been numerous reports of pathogenic fungi infecting other aphids, including *Neozygites fresenii* (Nowakowski) in the cotton aphid, *Aphis gossypii* Glover (Steinkraus *et al.* 1995, Hollingworth *et al.* 1995, Steinkraus *et al.* 1993a, Weathersbee & Hardee 1993, Steinkraus *et. al.* 1993b, Smith & Hardee 1993, Steinkraus *et al.* 1996) and the cereal aphids, *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (Linnaeus) & *Metopolophium dirhodum* (Walker) (Steenberg & Eilenberg 1995, Feng & Nowierski 1992); *Conidiobolous coronatus* (Costantin) in the cereal aphids, *S. avena, R. padi* and *M. dirhodum* (Steenberg & Eilenberg

1995), *A. gossypii* (Sanchez-Pena 1993), and the pea aphid, *Acyrthosiphon pisum* (Harris) (Brey & Latge 1986; Latge *et al.* 1986); *Beauveria bassiana* (Balsamo) in the three cereal aphids mentioned above and the hop aphid, *Phorodon humuli* (Schrank) (Feng *et al.* 1994); *Verticillium lecanii* (Zimmerman) in *A. gossypii* (Hall 1982; Sanchez-Pena 1993); aerial populations of *E. lanigerum* (Asante 1994), *S. avenae*, *R. padi* and *M. dirhodum* (Steenberg & Eilenberg 1995). The present study was initiated to determine whether or not pathogens or other potential biological control agents of subterranean *E. lanigerum* were to be found in the Western Cape Province of South Africa.

7.1 MATERIAL METHODS

7.1.1 Sampling. The sites used were described the Introduction.

7.1.1.1. *Soil samples*. One hundred aphids washed from the soil samples taken from the two blocks on each of the two farms described in Chapter 5, were selected and dissected and evidence of infection by pathogens was noted.

7.1.1.2. *Root samples*. Sixty trees were identified from block 2 on Molteno and from block 1 on Oak Valley. Five of these 60 trees were randomly selected on each sampling date. Infested roots were collected from

the five trees and transported to the laboratory in a cool bag. Sampling was carried out at two-weekly intervals. If a selected tree showed no signs of infestation, a tree next to it in an adjacent row not included in the original 60 trees was chosen. Aphids were washed from the roots into a sieve as described in Chapter 4. One hundred *E. lanigerum* were removed at random and dissected for pathogens.

In addition, 100 aphids from each orchard were randomly selected and sent to the National Collection of Fungi in Pretoria every month for isolation and identification of pathogens.

7.1.1.3. *Treatment of aphids.* *E. lanigerum* were dissected and examined for the presence of pathogens under a compound microscope using normal, phase contrast and dark field illumination. It was found that a mounting medium of a 0.01% solution of cotton blue stain in lactophenol facilitated the detection of fungi.

7.2 RESULTS

At least six species of entomopathogenic fungi were found in *E. lanigerum* (Table 7.1). In addition, at least 10 species of saprophytic fungi given in Table 7.1 may sometimes have a pathogenic effect on subterranean *E. lanigerum*. Besides the pathogenic fungi, at least 42 species of saprophytic

fungi were recorded (Table 7.2).

One mycoparasitic fungus, *Trichoderma sp.* was also reported in February 1999. There is at present confusion regarding the identity of *Hirsutella sp.* (Table 7.1). It may be *Engyodontium aranearum* which occurs in different spore forms, one of which closely resembles *Hirsutella sp.* Of the six pathogenic fungi identified by the National Collection of Fungi during 1997, 1998 and the first three months of 1999, *Hirsutella sp.* was the most frequently recorded (Table 7.1).

Of all the fungi given in Tables 7.1 and 7.2, only the genus *Conidiobolus* and *Hirsutella*, including *Hirsutella sp.,* could be identified in the *in situ* examination (Figs. 7.1 and 7.2).

Conidiobolus and *Hirsutella* were always active in aphids washed from soil and infested roots samples collected at Molteno and Oak Valley (Fig. 7.1A, B and 7.2A, B).

Conidiobolus. On Molteno during 1997 the highest level of *Conidiobolus* infection in soil and root samples was in December (Fig. 7.1A and B). In 1998 there were two infection peaks, one at the beginning of February and a second in December (Fig. 7.1A and B). On Oak Valley peak infection levels of *Conidiobolus* in the soil and root samples occurred in November during 1997 (Fig. 7.2A and B), in June during 1998 in the soil

samples and at the end of May and beginning of January 1999 (Fig. 7.2A and B) in the root samples.

Hirsutella. On Molteno during 1997 the highest level of *Hirsutella/Engyodontium* infection in the soil and root samples occurred in September, and in 1998 peak infection levels were recorded in November which was earlier than the *Conidiobolus* infection peak (Fig. 7.1A and B). In addition, in the root samples high infection levels were also recorded during January 1999 (Fig. 7.1B). In Oak Valley soil samples during August 1997 almost 50% of the subterranean *E. lanigerum* were infected with *Hirsutella/Engyodontium*. From August 1997 until April 1998 infection levels always exceeded 5%. After April this increased and peaked at more than 40% infection during August 1998, with the lowest infection levels occurring at the end of September 1998 (approximately 2-3%) (Fig. 7.2A). In the root samples peak infection levels in 1997 occurred at the end of July and beginning of August. During 1998 there were some fluctuations. The first infection peak occurred at the beginning of February, it then declined until April but increased again during May (Fig. 7. 2B).

Generally, fungal infection levels in aphids from root and soil samples taken from July until the end of December in both orchards were higher in 1997 than at the same time in 1998 (Figs. 7.1A, B and 7.2A, B).

Table 7.1. Pathogenic fungi identified by the National Collection from subterranean *Eriosoma lanigerum* samples taken at regular intervals during 1997, 1998 and first three months of 1999.

	1997											1998												1999		
FUNGUS	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**
Acremonium implicatum (Gillman & Abott)*																		X								
Aspergillus niger Van Tieghem*																			X							
Beauvaria bassiana (Bals.)				X														X					X			
Chaetomium funicolum Cooke*																			X							
Cladosporium sp.*						X	X					X	X	X	X			X	X	X			X	X	X	X	X
Conidiobolus coronatus (Constatin)	X	X																					X			
Conidiobolus obscurus (Hall & Dunn)	X																									
Engyodontium album (Lumber)*	X	X																					X			
Fusarium oxysporum Schltdl*				X				X	X	X		X	X		X				X			X				
Gliocladium roseum Bainier*				X	X	X		X	X	X		X	X		X							X				
Hirsutella illustris Minter & Brady						X													X							
Hirsutella sp.							X		X			X	X	X	X		X	X	X	X	X	X	X		X	X
Neozygites fresnii (Nowakowski)	X	X																								
Paecilomyces lilacinus (Thom)*																						X		X		
Sporothrix sp.*							X																			
Verticillium lecanii (Zimm.)				X					X										X							
Verticillium psalliotae Treschow*								X			X								X	X						
Verticillium sp.*								X		X	X								X							

* Saprophytic fungi also reported which sometimes could have a pathogenic affect.

Table 7.2. Saprophytic fungi identified by the National Collection from subterranean *Eriosoma lanigerum* samples taken at regular intervals during 1997, 1998 and first three months of 1999.

	1997											1998												1999		
FUNGUS	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M
Acremonium sclerotigenum (Moreau & R. Moreau & ex Vallenta)																				X						
Acremonium sp.																				X					X	
Acremonium strictum W. Garns																	X									
Acremonium terricola (Miller & al.)													X						X						X	
Alternaria sp.						X																				
Alterneria alternata (Fr.)													X										X	X		
Aspergillus japonicus Saito				X																						
Aspergillus sp.														X												
Aureobasidium pullulans var. melanginium													X													
Aureobasidium sp.																			X							
Bipolaris sp.																										X
Chaetomium sp.												X														
Cladosporium cladosporioides (Fresen.)										X																
Cylindrocarpon sp.											X															
Diplodia mutila Fr.																				X						
Epicoccum nigrum Link																								X		
Epicoccum sp.																									X	X
Fusarium crookwellense Burgess																	X									
Fusarium equiseti (Corda)						X																				
Fusarium solani (Mart.)								X		X																
Fusarium sp.														X	X		X	X					X	X		X

Table 7.2. (continued)

FUNGUS	1997 F	M	A	M	J	J	A	S	O	N	D	1998 J	F	M	A	M	J	J	A	S	O	N	D	1999 J	F	M
Gilmaniella subornata Morinaga																				X						
Gliocladium catenulatum Gill. & Abbott.								X																		
Gliocladium penecilloides Corda											X															
Gliocladium sp.														X												
Gliomastix murorum (Corda)						X			X																	
Mariannaea elegans (Corda)																	X								X	
Mucor circinelloides Tiegh.																	X									
Mucor sp.														X												X
Myrothecium verrucaria (Alb. & Schw.)																								X		
Paecilomyces inflatus (Burnside)																	X									
Paecilomyces sp.																				X						
Paecitomyces variolii Bainier				X		X																				
Penicillium citrinum Thom				X		X																				
Penicillium olsonii Bainier & Sartory				X		X																				
Penicillium spp.					X	X		X		X																
Periconia igniara E. W. Mason & M. B. Ellis																				X						
Pestalotiopsis sp.												X														
Phialophora sp.								X																		
Phoma sp.								X																		
Ramichloridium schulzeri (Sacc.)												X										X				
Rhizomucor pusillus (Lindt) Schippet								X																		
Rhizopus microsporus van Tieghem																									X	
Rhizopus sp.																			X							
Torula sp.																										X
Trichoderma harzianum Rifai																				X						
Truncatella angustata (Pers. Link)																	X							X		
Verticillium fungicola (Preuss.)																	X									
Verticillium tenerum (Nees ex Pers.)																								X		

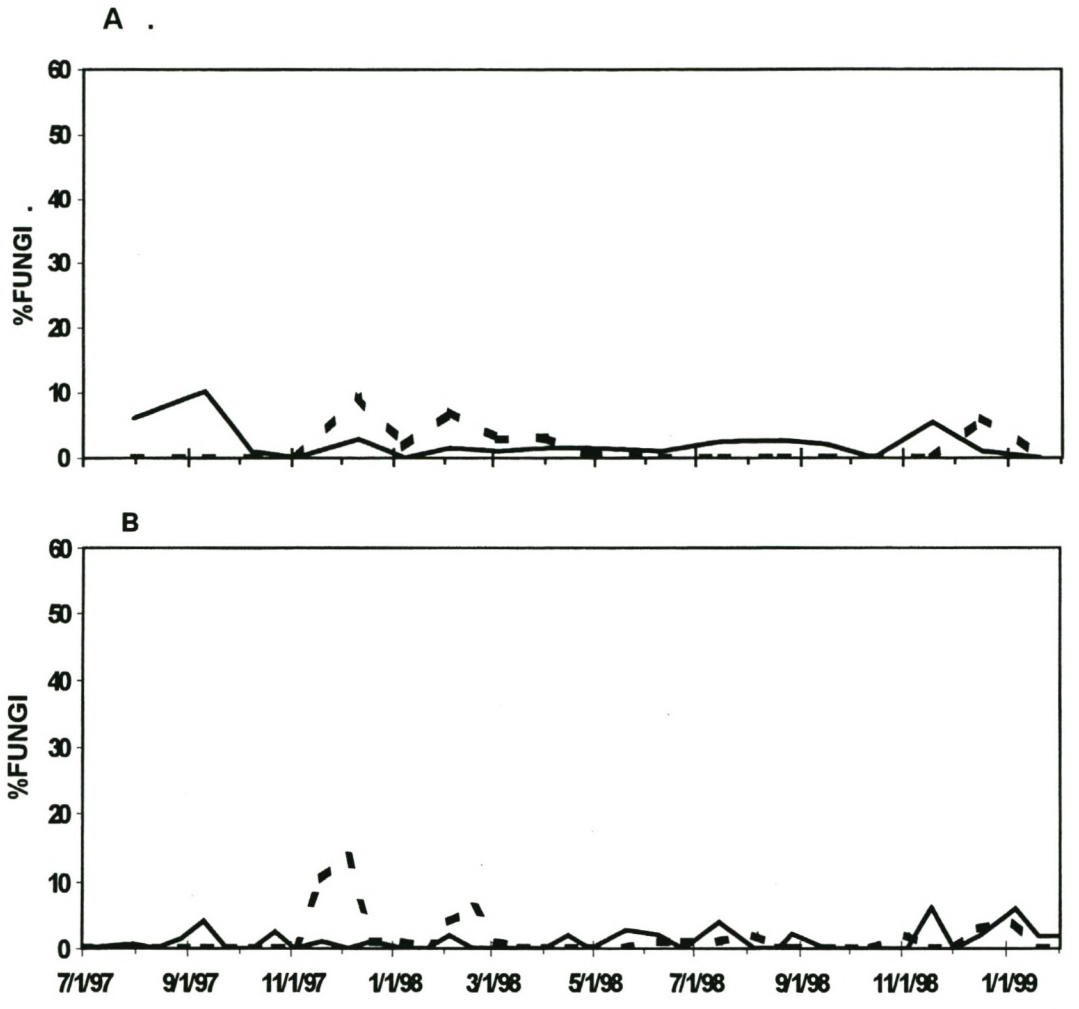

Fig. 7.1. Percent *Eriosoma lanigerum* infected by *Conidiobolus* (thick broken line), *Hiesutella/Engyodontium* (thick line) from Molteno. A: aphids washed from soil samples; B: aphids washed from root samples.

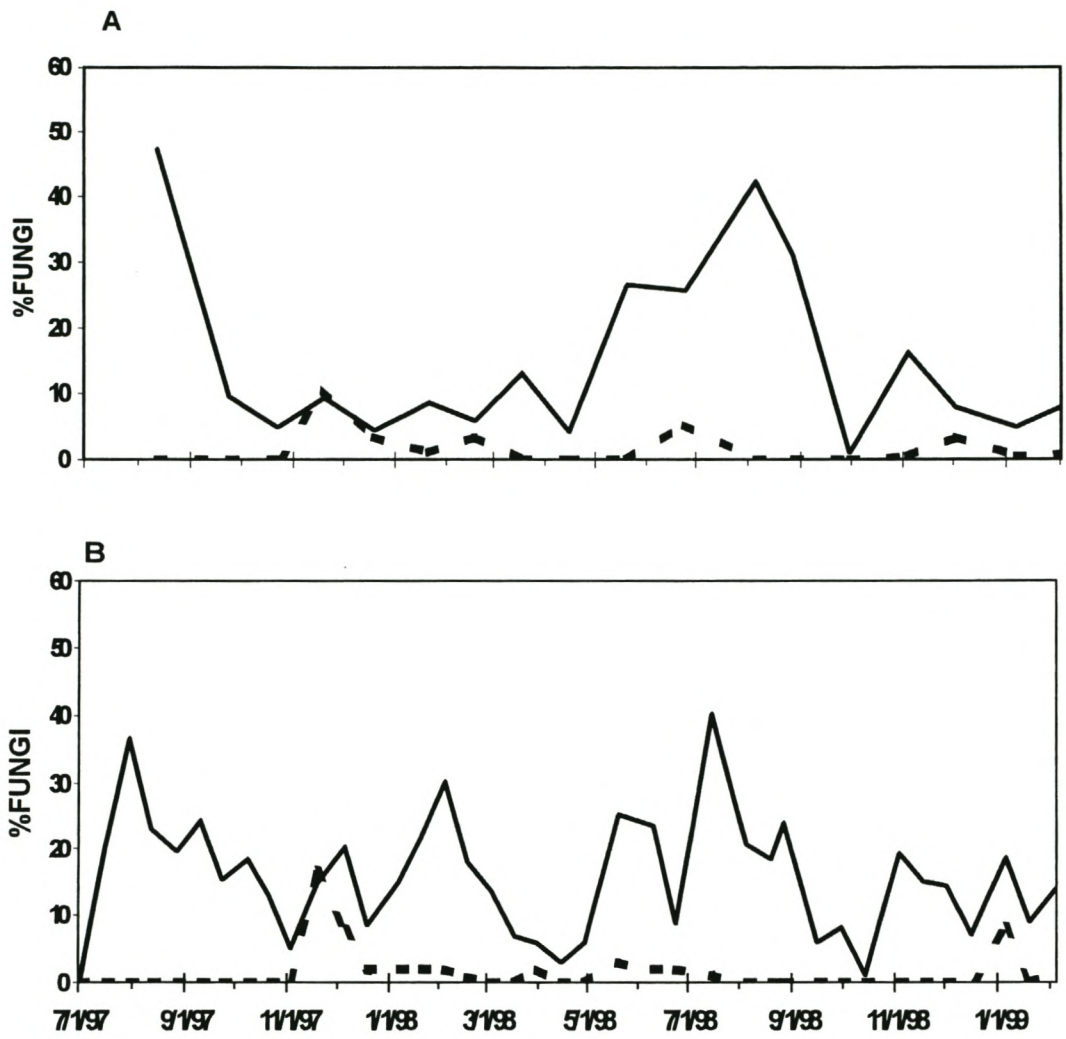

Fig. 7.2. Percent *Eriosoma lanigerum* infected by *Conidiobolus* (thick broken line), *Hirsutella/Engyodontium* (thick line) from Oak Valley. A: aphids washed from soil samples; B: aphids washed from root samples.

7.3 DISCUSSION

The higher infection levels on both sites during August to December 1997 in comparison with the same period in 1998 (Figs. 7.1. A. B and 7.2. A. B.) can possibly be attributed to the higher rainfall during these months in 1997 (Fig. 3). It is possible that rainfall increased fungal infection levels during 1997 since high humidity is considered vital for the germination of fungus spores and transmission of the pathogen (Metcalf & Luckmann 1982).

Hirsutella/Engyodontium and *Conidiobolus spp.* were active throughout the year at Molteno and Oak Valley. The highest peak of fungal infection at Oak Valley occurred during August 1997 in soil samples and affected almost 50% of the subterranean *E. lanigerum* population (Fig. 7.2A). At Molteno highest infection levels were during September and more than 10% of the subterranean *E. lanigerum* in soil samples were infected (Figs. 7.1A). Overall on Molteno the infection by *Hirsutella/Engyodontium* in both root and soil samples was lower than on Oak Valley, while infection by *Conidiobolus spp.* in both orchards seemed to be similar (Figs. 7.1A, B and 7.2A, B). However, on Oak Valley *Hirsutella/Engyodontium* infection was obviously higher than that of *Conidiobolus spp.* (Fig. 7.2A, B) while on Molteno *Hirsutella/Engyodontium* and *Conidiobolus*

spp. infection were similar (Fig. 7.1A, B).

Subterranean *E. lanigerum* populations were infected by a wide variety of pathogenic fungi (Table 1), which may reduce their numbers. Some of them could be useful in the management of this species. However, the vast array of saprophytic fungi present makes it very difficult to directly determine the relative importance of individual pathogenic fungi. Therefore, pure isolates of the pathogens will have to be screened for pathogenicity to *E. lanigerum*.

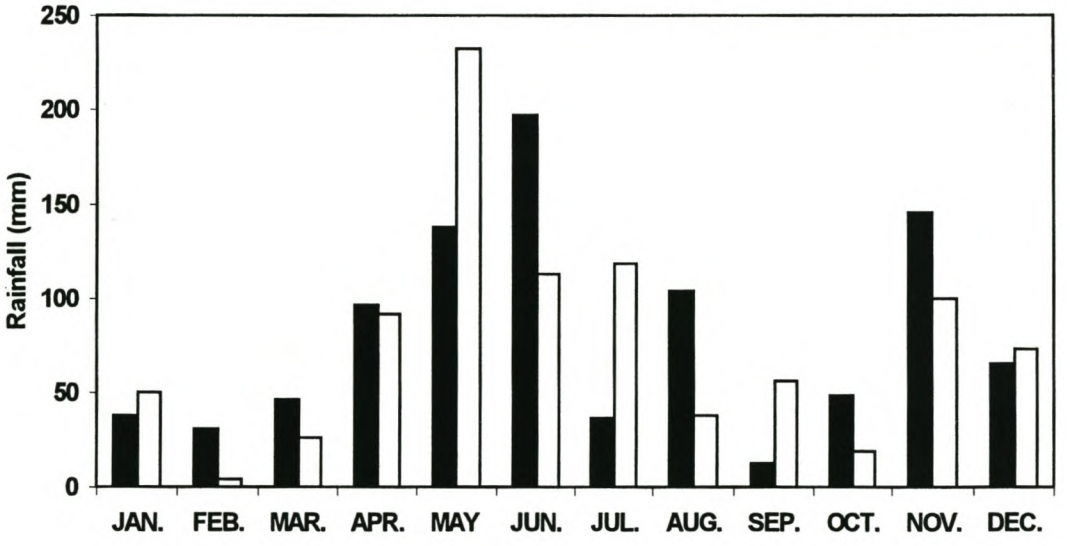

Fig. 7.3. Total monthly rainfall for 1997 (shaded bars) and 1998 (clear bars) Elgin.

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CHAPTER 8

EFFECTS OF STRAW MULCHES ON SUBTERRANEAN *ERIOSOMA LANIGERUM* POPULATIONS

In the past various attempts to control underground populations of *Eriosoma lanigerum* (Hausmann) have been made. Fuller (1904) applied near boiling water around the trunk of the tree and for improved results, he added tobacco dust. Greenslade (1936) applied grease bands to the trunks to trap the crawlers on their migration down to the roots. A wide variety of pesticides have also been used in apple orchards to control subterranean *E. lanigerum* (Lohrenz 1911, Greenslade 1936, Smith 1942, Stanly 1951, Attri & Sharma 1971, and Garg *et al.* 1977, Pringle 1998).

Apple rootstocks resistant to *E. lanigerum* have been identified (Staniland 1924, Knight *et al.* 1962, Rock & Zeiger 1974, Taylor 1981, Young *et al.* 1982). However, Giliomee *et al.* (1968) found a strain of the aphid which had overcome the resistance factor in Northern Spy and related rootstocks in South Africa. In South Africa at present the only control measure available for subterranean populations of *E. lanigerum* is a soil application of imidacloprid (Pringle 1998). This is tedious and

expensive.

During the course of basic studies (Chapter 4) of this insect, it was observed that when the soil became saturated after the onset of the winter rains, subterranean populations declined. In addition, these studies revealed the existence of a number of entomoparasitic fungi (see Chapter 7). These fungi appear to require high humidity levels (Odour *et al.* 1996a, Beyer *et al.* 1997a, Odour *et al.* 1995, Steinkraus & Slaymaker 1994). In addition, it has been reported that high levels of humus material favour the survival of *Verticillium lecanii* (Zimermann) (Beyer *et al.*1997b). Different reports have also indicated that darkness and cool temperatures (13 to 23 ^{0}C) stimulated sporulation and germination of the conidia of some of entomopathogenic fungi (Odour *et al.* 1996a, Odour *et al.* 1996b, Uziel & Kenneth 1991). Therefore, the purpose of the present study was to examine whether or not underground *E. lanigerum* populations would decline after the application of a straw mulch which would keep the soil moist and cool and increase levels of humus at the soil surface.

8.1 MATERIAL AND METHODS

An orchard of Granny Smith and Top Red trees planted in 1985

on M 793 rootstock was used. There were four rows of Granny Smith alternated with two of Top Red. Only the Granny Smith trees were used. The rows contained between 60 and 70 trees and one row was used per replicate. There were three treatments. They were imidacloprid (Confidor®) applied on 4 December 1997 as described by Pringle (1998), straw applied at approximately 5 kg/m in a strip of about 0.5 m on either side of the trees on 25 August 1997 and an untreated control.

The experiment was laid out in a totally randomised (one-way) design replicated five times. Soil samples were taken every two weeks and colonies were counted in the aerial parts of the trees at intervals of one to three weeks. The soil samples were taken using the hand auger described in Chapter 4. They were taken 30 cm from the northern side of the trunk of six trees per replicate.

There were eight trees between each sample tree. Samples were taken from different trees on each sampling date to avoid multiple sampling from the same point. Pre–treatment samples could not be taken because sampling was destructive and the disturbance of the soil and removal of roots would affect results obtained from future sampling.

The subterranean *E. lanigerum* specimens were washed out of the soil through a 200 µ sieve as described in Chapter 4. A distinction was

made between dry, dead and alive aphids. The dry aphids were assumed to have died prior to sampling and were excluded from the data used in the analyses. The dead ones were still turgid and were assumed to have died during the sampling and washing process. Therefore, they were included in the data used in the analyses.

Colonies were counted in the leaf axils on the northern half of each of 25 trees per replicate up to chest height. The counts were conducted on every second tree and a distinction was made between parasitised and unparasitised colonies. A colony was considered to be parasitised if at least one individual in the colony was parasitised. The total number of colonies was used in the analyses.

The data were analysed using a one-way analysis of variance after log transforming to stabilise the variances. Means were compared using Bernoulli's inequality to compensate for multiple comparisons.

In addition, the apple yield in the three treatments was determined during the season following the treatment, because root infesting woolly apple aphids would affect the reserves required for producing the following season's crop (Titus & Seong-Mo Kang 1986). These data were analysed using an analysis of variance.

One hundred *E. lanigerum* washed from the soil samples as

described above were removed at random and dissected for pathogens. The data were analysed as a randomised block design with date as blocks.

8.2 RESULTS

The underground *E. lanigerum* populations were lower in the straw treatment than in the control on all the sampling dates (Table 8.1). On four of the nine sampling dates these differences were significant (18/12/97, 29/12/97, 14/1/98 and 8/4/98) (Table 8.1). In addition, the subterranean *E. lanigerum* populations were lower in the straw than in the imidacloprid treatment on five of the nine sampling dates (18/12/97, 11/2/98, 11/3/98, and 8/4/98) (Table 8.1). However, only one of these differences was significant and this occurred on the first sampling date (18/12/97). Fewer *E. lanigerum* were counted in soil samples from the imidacloprid treatment than in those from the control on all the sampling dates (Table 8.1). On two of the nine sampling dates the differences were significant (29/12/97 and 14/1/98).

There were more infested leaf axils in both the control and imidacloprid treatments than in the straw treatment on all sampling dates (Table 8.1 and Fig. 8.1). The differences between the control and straw

treatments were significant on all the sampling dates (Table 8.1), while the differences between the imidacloprid and straw treatments were significant on all dates except three (14/1/98, 18/3/98 and 15/4/98).

Apple production per tree in the imidacloprid treatment was higher than in the straw treatment and in the control (Fig. 8.2). However, these differences were not significant ($F_{2:12} = 2.43$; $P = 0.13$).

Beauveria was only recorded on two sampling dates (29/12/1997 and 14/1/1998) and *Hirsutella* on three (4/1/1998, 11/2/1998 and 11/3/1998). Therefore, the data for these fungi were not analysed, although they are included in the graph (Fig. 8.3). The most commonly recorded entomopathogenic fungus was *Conidiobolus*. More aphids infected with *Conidiobolus* were found in the imidacloprid treatment than in the control or straw mulch treatments (Fig. 8.3). However, these differences were not significant ($F_{2:12} = 1.78$; $P = 0.211$). Quintela & McCoy (1997) indicated that imidacloprid appeared to enhance the pathogenicity of some entomopathogenic fungi occurring in the soil.

8.3 DISCUSSION

As discussed in Chapter 5 higher rainfall could have suppressing effect on the subterranean *E. lanigerum* population level by increasing

soil moisture. Some authors have shown that humidity levels increase under the mulches (Hartley *et al.* 1996 & Merwin *et al.* 1994). Therefore, it was thought that straw increased the soil humidity thereby reducing edaphic *E. lanigerum* numbers. It appeared as if the suppressing effect of straw on the underground *E. lanigerum* populations was similar to that of imidacloprid. However, there were fewer infested leaf axils in the straw treatment than in the imidacloprid treatment, suggesting that the straw also interfered with the ability of the crawlers to enter the trees

High humidity is considered vital for the germination of fungus spores and transmission of fungal pathogens (Metcalf & Luckmann 1982). However, in the present study there was no evidence of increased fungal infection under the straw mulch.

The level of control of the subterranean populations using a straw mulch was at least as good as that provided by imidacloprid, while there were fewer crawlers infesting the leaf axils in the straw treatment than in the imidacloprid treatment. Therefore, straw mulch may provide an alternative to the soil insecticide, imidacloprid.

Table 8.1. Average *Eriosoma lanigerum* per soil core and *E. lanigerum* colonies per tree on nine sampling dates in untreated control plots, plots treated with imidacloprid and plots treated with straw mulch. Counts of aphids per soil core and counts of colonies per half tree in the three treatments not followed by the same letter on a given date differed at the 5% significance level.

	Aphids per soil core			Colonies per half tree		
Date	Control	Imidacloprid	Straw	Control	Imidacloprid	Straw
18/12/97	28.20 a	13.58 a	0.14 b	36.18 a	26.38 a	5.65 b
29/12/97	67.37 a	4.47 b	6.07 b	7.31 a	7.18 a	1.36 b
14/1/98	84.10 a	6.03 b	7.77 b	5.79 a	9.58 ab	0.48 b
26/1/98	37.53 a	2.53 a	6.98 a	8.96 a	7.76 a	0.64 b
11/2/98	56.27 a	12.33 a	7.60 a	6.08 a	4.06 a	0.66 b
25/2/98	30.30 a	2.92 a	28.90 a	11.75 a	5.71 a	1.86 b
11/3/98	37.53 a	4.53 a	2.43 a	-	-	-
18/3/98	-	-	-	16.98 a	7.42 ab	2.70 b
25/3/98	47.77 a	5.80 a	4.27 a	21.72 a	13.72 a	3.71 b
8/4/98	47.60 a	11.33 ab	2.30 b.	-	-	-
15/4/98	-	-	-	56.30 a	22.82 ab	14.76 b

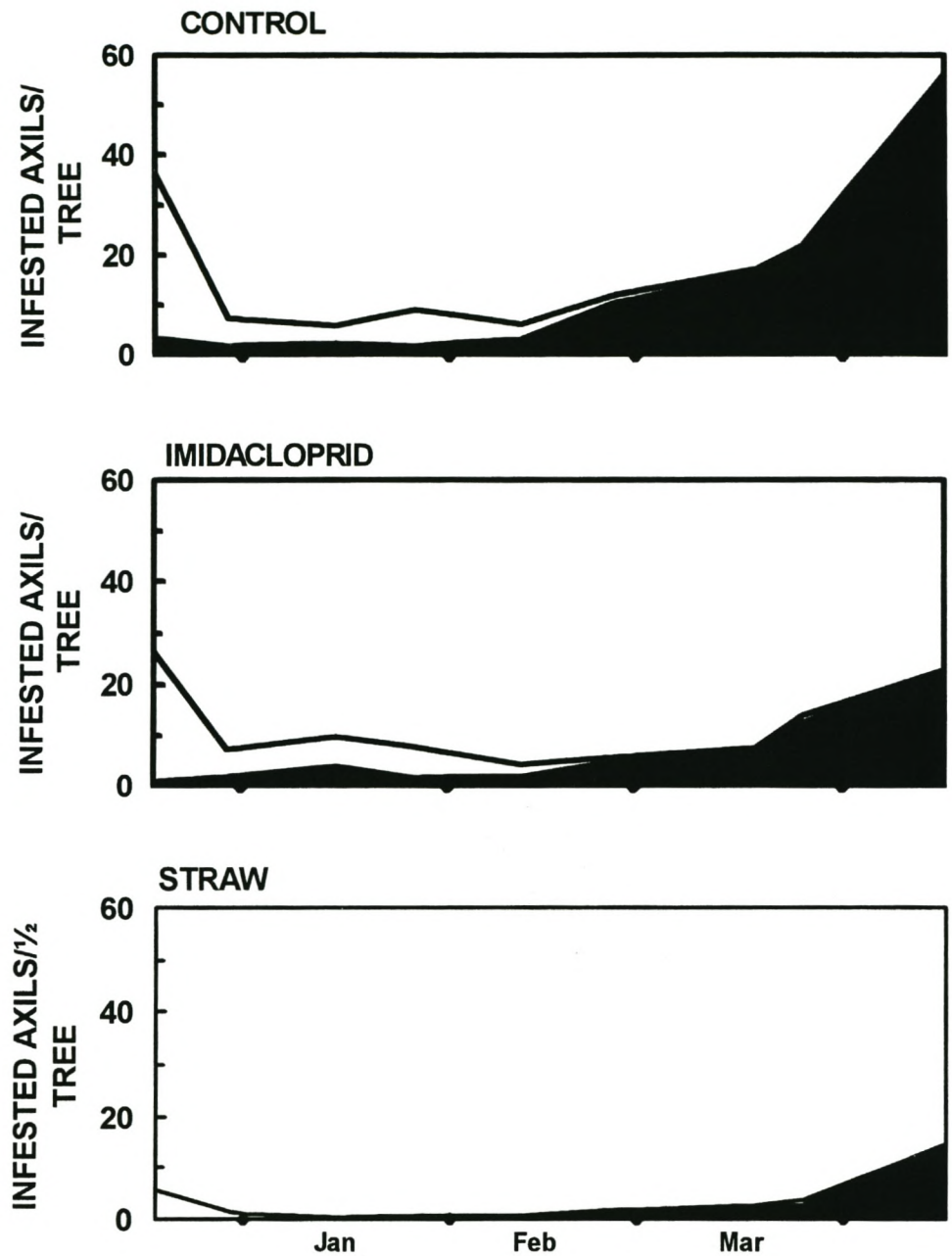

Fig. 8.1. Average number of leaf axils per half tree infested with parasitised *Eriosoma lanigerum* colonies (shaded area) and parasitised plus unparasitised colonies (clear area).

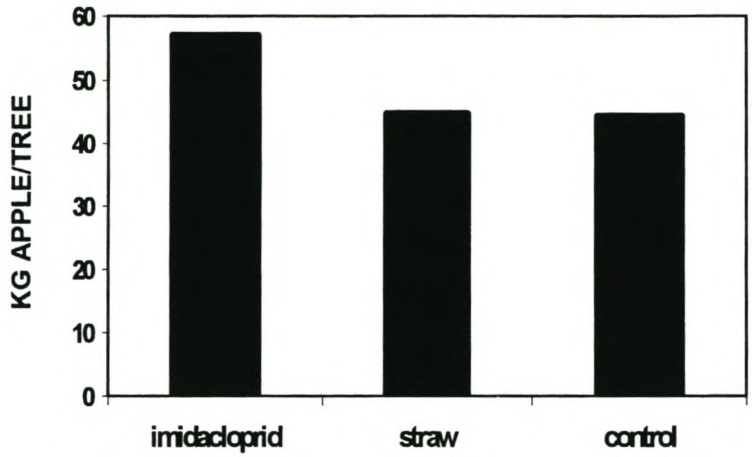

Fig. 8.2. Average fruit yield per tree treated during the previous season with imidacloprid and straw mulch.

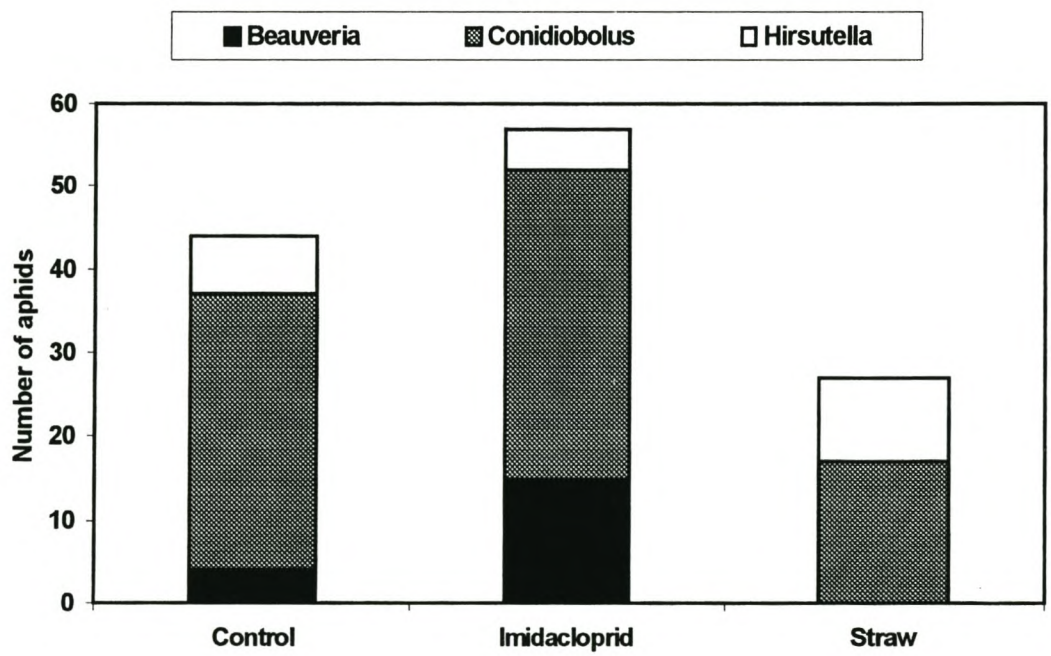

Fig. 8.3. Number of *Eriosoma lanigerum* infected with entomopathogenic fungi in control, imidacloprid and straw mulch treatments.

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CHAPTER 9

GENERAL CONCLUSIONS

A study of the biology of subterranean *Eriosoma lanigerum* (Hausmann) was conducted. The first instar could be identified by the absence of cornicles and adults by the presence of a vulva. In an attempt to distinguish between instars 2, 3 and 4, thirteen structures were measured. Of these only three were suitable. They were body length, body width and length of the hind femur. However, it was not possible to distinguish between the 2nd and 3rd instars with confidence. There was a marked increase in body size (body length and width) in the 4th instar.

The effects of temperature on development and reproduction were determined. The most suitable temperature for the development of subterranean *E. lanigerum* was 23 °C, while the lower and upper thresholds were 4.32 and above 30 °C, respectively. The effects of soil moisture on population development appeared to be important, and requires further investigation.

Regular sampling of underground *E. lanigerum* to determine cyclical, seasonal changes in population levels proved difficult. Aphid numbers in soil core samples were not only determined by the underground population levels, but also by the distance at which samples were taken from the trunk, as well

as the presence and nature of root material in the samples. The distance from the trunk could be standardised, but there was no control over the root material in the sample, which introduced an unavoidable source of sampling error. Because of these difficulties, this sampling system will not be suitable for making decisions on whether or not a soil treatment for the control of underground *E. lanigerum* populations should be applied. Such a system, based either on damage symptoms on the roots or on the level of infestation of the aerial parts of the trees, needs to be developed.

All developmental stages of *E. lanigerum* were present on the roots of apple trees throughout the year. However, there appeared to be two peaks in numbers of subterranean aphids, one during early summer and one during autumn. With the onset of winter rain, *E. lanigerum* population levels declined. However, during the winter of 1997 this was not so obvious. Since the rainfall during the 1997 winter was lower than during the other two winters, it was speculated that soil moisture reduced underground population levels.

The early and late summer increase in subterranean *E. lanigerum* population levels coincided with peak levels of nitrogen in the roots, but it was not clear whether or not there was a causal relationship. However, if there was a causal relationship, nitrogen is actively taken up by the roots during autumn (Titus & Seong Mo Kang 1986). Therefore, nitrogen is applied at this

time of the year, and there is no scope for manipulating the timing of these applications to suppress woolly apple aphid population levels without adversely affecting the nutritional status of the tree.

Crawler movement into the aerial parts of the trees coincided with the early summer population increase, but not with the late summer increase. There is at present no explanation for this.

Embryos were present in all instars throughout the year. However, fourth instar and adult aphids contained more embryos than the other instars. This could probably explain why body length and width provided good separation between the 3rd and 4th instars. The maximum number of embryos in the 4th instar and adult aphids occurred during spring (September to October), which coincided with the early season peak in nitrogen levels. However, it was clear that nitrogen level was not the only factor which affected the number of embryos, because during winter there was a higher total number of embryos, due to higher numbers of embryos in the first three instars. In addition, nitrogen levels at Molteno were consistently higher than at Oak Valley, while there appeared to be more embryos in aphids from Oak Valley than in those from Molteno. The trees at Oak Valley were on seedling rootstocks while those at Molteno were on M793. Therefore, fluctuations in nitrogen levels in the roots may help to explain fluctuations in seasonal

production of embryos and fluctuations in population levels, but the overall fecundity and numbers of subterranean *E. lanigerum* populations appeared to be determined by other factors

A variety of entomopathogenic fungi infecting subterranean *E. lanigerum* were identified. These included species of *Conidiobolus*, *Beauveria* and *Hirsutella*, all well known entomopathogens. The potential for biological control of underground woolly apple aphids using these fungi needs to be researched.

A straw mulch suppressed subterranean *E. lanigerum* populations at least as well as the registered soil insecticide, imidacloprid. In addition, it appeared as if the straw mulch interfered with the movement of crawlers into the aerial parts of the trees. The suppression of subterranean woolly apple aphid population levels by the straw mulches was thought to be the result of an increase in soil moisture due the mulch application. Therefore, any orchard practices which increase moisture levels in the soil, such as manipulating irrigation regimes so as to maintain reasonably high levels of. soil moisture, may reduce subterranean populations. This approach for the control of subterranean *E. lanigerum* needs further investigation.

With this study a contribution has been made towards understanding the basic biology of the subterranean populations of *E. lanigerum*. This can

provide a basis for planning the management of this pest of apple tree roots in the future. Fruitful areas of future research, besides increasing soil moisture levels, would be towards exploiting entomopathogenic fungi for the control of underground woolly apple aphid populations.

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