ASPECTS OF THE BIO-ECOLOGY OF THE BITING LOUSE, *DAMALINIA LIMBATA*

by

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OPSOMMING

Bytende luise is bekende ektoparasiete van vertebrate. Meeste wilde en mak- of huisdiere het een of meer luisspesie wat hulle parasiteer. Alhoewel die luise 'n bron van irritasie vir die gasheer is, word hulle nie normaalweg as ekonomies belangrik beskou nie, aangesien hulle min fisiese skade veroorsaak. Daar is wel aanduidings dat die bytende skaapluis (*Damalinia ovis*) verliese in wolproduksie en kwaliteit kan veroorsaak. *Damalinia limbata*, wat Angora bokke infesteer, kan moontlik dieselfde tipe verliese veroorsaak as *D. ovis* op skape. Min navorsing is egter nog gedoen op hierdie luise en gevolglik kan die invloed wat hulle moontlik op sybokhaar-produksie en -kwaliteit het nie objektief bepaal word nie.

Die doelstellings van hierdie studie was om aspekte van die biologie en verspreiding van *D. limbata* te ondersoek sowel as die bevordering van effektiewe en ekonomiese bestuur van die luise op plase. Die volgende is ondersoek: (1) Aspekte van *D. limbata* en *D. ovis* se morfologie. (2) Die omgewings temperature op die lyf van Angora bokke. (3) Die aantal nimf instars van *D. limbata*. (4) Die seisoenale veranderings in *D. limbata* bevolkings. (5) Die invloed van *D. limbata* op die liggaamsmassa van Angora bokke en die invloed op bokhaarproduksie en -kwaliteit asook die doeltreffendheid van verskillende beheermetodes.

Veldwerk is op die plaas Preezfontein (29°50'S, 25°19'E) ongeveer 10 km buite Fauresmith in die suid-wes Vrystaat (ongeveer 130 km suid-wes van Bloemfontein) gedoen. Die veld tipe in hierdie area word beskryf as Skyn Hoër Karoo en val binne die Karoo bioom. Die Vrystaat is 'n somer-reënval gebied met jaarlikse neerslag van ongeveer 450-500 mm. Somers in die gebied is baie warm en die winters koud, met droogtes wat gereeld voorkom.

Die algemene morfologiese kenmerke, morfometriese kenmerke en die plasing van dorsale en ventrale abdominale plate van *D. limbata* en *D. ovis* is vergelyk. *D. limbata* vertoon stewiger in vergelyking met *D. ovis*. Wyfies van beide *D. limbata* en *D. ovis* is ongeveer 1.611 mm lank en die mannetjies was onderskeidelik 1.378 mm en 1.255 mm lank. Manlike *D. ovis* het soliede

dorsale plate vergeleke met *D. limbata* wat dorsale plate het wat op segmente IV en V transversaal gesplete is.

Temperatuur studies op die mikro-habitat van *D. limbata* het getoon dat temperature teen die vel van Angora bokke relatief konstant bly teen ongeveer 35 °C. Alle pogings om 'n laboratorium kolonie te begin, het misluk.

D. limbata het drie nimf stadia. Instar 1 het 'n kopkapsule met 'n gemiddelde lengte en breedte van onderskeidelik 0.202 mm en 0.252 mm. Instar 2 se kopkapsule lengte en breedte was onderskeidelik 0.305 mm en 0.364 mm en die van instar 3 was onderskeidelik 0.467 mm en 0.425 mm.

Luis bevolkings het gedurende lente en vroeg-somer toegeneem met 'n piek in die middel van die somer. Skeertyd in die winter het blykbaar 'n groter invloed op luis getalle as gedurende die somer. Die luise beweeg rond op die bokke en is meer volop op die ventrale gedeeltes van die liggaam gedurende die somer. Gedurende die winter is die luise meer eweredig oor die liggaam van die bokke versprei.

D. limbata het nie 'n negatiewe invloed op die liggaamsmassa van Angora bokke nie. Daar is wel 'n negatiewe invloed in bokhaarproduksie en -gehalte waargeneem. Die gemiddelde verlies in bokhaarproduksie was 12 % en op sommige bokke was dit so hoog as 25 %, wat redelike ekonomiese verliese vir die produsent verteenwoordig. *D. limbata* word effektief deur Deltamethrin beheer wanneer dit as 'n dorsale behandeling toegedien word, of as 'n laterale toediening met 'n Tikspray toediener.

ABSTRACT

Biting lice are well known ecto-parasites of vertebrates. Most wild and domesticated animals have one or more louse species, living on them. Although lice are a source of irritation to the host, they are not generally considered as economically important because they do not cause much physical damage. The sheep biting louse (*Damalinia ovis*) has, however, been shown to cause losses in wool production and quality. *Damalinia limbata* are ecto-parasites on Angora goats and can cause the same type of losses to the farmer as the sheep biting louse. Very little research has been done on these lice and their impact on mohair production can therefore not be objectively assessed.

The objectives of the current study were to investigate aspects of the biology and distribution of *D. limbata* and to promote more effective and economical management of these lice on commercial farms. The following were investigated: (1) Aspects of the morphology of *D. limbata* and *D. ovis*. (2) Environmental temperatures prevalent on the body of Angora goats. (3) The number of nymphal instars of *D. limbata*. (4) Seasonal changes in the populations of *D. limbata*. (5) The influence of *D. limbata* on the body mass of Angora goats and the production and quality of mohair, as well as the efficacy of different control methods.

Field experiments were conducted on the farm Preezfontein (29°50'S, 25°19'E), situated 10 km from the town Fauresmith, about 130 km southwest of Bloemfontein in the south-western Free State. The veld type of this area is defined as 'False Upper Karoo' and falls in the Karoo biome. The Free State is a summer rainfall region with an average precipitation of 450-500 mm per annum, with hot summers and cold winters and droughts occurring regularly.

D. limbata and *D. ovis* were compared using general morphological characters, morphometric measurements of various body regions and placement of dorsal and ventral abdominal sclerites. *D. limbata* has a more robust appearance than *D. ovis*. Females of both *D. limbata* and *D. ovis* were on average 1.611 mm long and the males had average lengths of 1.378 mm and 1.255 mm,

respectively. Male *D. ovis* had solid dorsal plates where as *D. limbata* males had dorsal plates, which were transversally split, on segments IV and V.

Temperature studies, on the micro-habitat of *D. limbata*, showed that the temperature against the skin of an Angora goat is relatively constant at approximately 35 °C. All attempts to establish a laboratory colony of *D. limbata* were unsuccessful.

D. limbata was found to have three nymphal instars before reaching adulthood. Instar 1 had average head-capsule widths and lengths of 0.252 mm and 0.202 mm, respectively. The second and third instars had head-capsule widths of 0.364 mm and 0.467 mm and lengths of 0.305 mm and 0.425 mm respectively.

The louse populations increased during spring and early summer, peaking in mid summer. Mid winter shearing seemed to have a greater impact on the louse populations than mid summer shearing. *D. limbata* moves around the body of Angora goats, being more abundant on the ventral areas of the body during summer and more evenly dispersed over the body during winter.

It was found that *D. limbata* does not have an adverse influence on the body mass of Angora goats, but does adversely affect mohair production and quality. The average loss in mohair production was 12 % and individual losses of as much as 25 % were recorded, representing substantial financial losses to the farmer. *D. limbata* was effectively controlled by Deltamethrin when applied as either a backline treatment or as a lateral application with a Tikspray applicator.

Key words: Phthiraptera; *Damalinia limbata*; lice; Angora goats; temperature; instars; mohair; body mass; mohair mass; chemical control.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION.

The superorder Psocodea is composed of two orders, namely the Psocoptera (booklice and barklice) and the Phthiraptera (lice) (Lyal, 1985a). The Psocoptera are free-living insects feeding on fungi or fragments of animal or vegetable matter. Some are associated with mammals or birds by inhabiting their burrows or nests, but none are parasitic (Lyal, 1985a). Phthirapterans, on the other hand, have no free-living stages and they are all obligate ectoparasites of mammals and birds (Ledger 1980, Lyal, 1985a; Baker, 1994).

It is generally recognized that the Phthiraptera can be divided into four major groups, namely the Anoplura, Rhyncophtherina, Ischnocera and Amblycera (Lyal, 1985a; Baker, 1994). The Anoplura are commonly known as sucking lice, because most are blood feeders (Lyal, 1985a; Baker, 1994). The other three (sometimes collectively referred to as the Mallophaga) are commonly known as chewing lice, because they feed on skin-scruff, feathers and sebaceous excretions, none penetrating the skin of the host (Ledger, 1980; Lyal, 1985a; Baker, 1994). The phylogenetic relationships of these four groups and the way they should be classified are, however, matters of some contention (Ewing, 1936; Lyal, 1985a, Baker, 1994)

The biting lice, found on Angora goats, are classified according to Ledger (1980); Lyal (1985a) and Baker (1994) as follows:

Phylum : Arthropoda
Class : Insecta
Superorder : Psocodea
Order : Phthiraptera
Suborder : Ischnocera
Family : Trichodectidae
Genus : *Damalinia* (*Bovicola*?)
Species : *limbata*

The genus, *Bovicola* Ewing 1929, was considered as a subgenus of *Damalinia* Mjoberg 1910 by Hopkins (1949), Hopkins & Clay (1952) and Ledger (1980). Some other workers in the field (Ewing, 1936; Emerson & Price, 1981 and Lyal, (1985b) have given *Bovicola* full generic status. The present generic status of most Trichodectidae is still, however, somewhat uncertain. The present study follows Ledger (1980) in accepting *Damalinia* as the genus for the species studied.

The Angora goat originates from Asia Minor and owes its name to the geographical area of Angora (Terblanche, 1979). The first Angora goats were introduced into South Africa in 1838, when Colonel Henderson imported one ewe and twelve rams from Turkey (Terblanche, 1979). Further imports of goats in small numbers continued between 1857 and 1869 with larger herds being imported afterwards. These imports continued until the Sultan of Turkey placed a ban on further Angora goat exports in 1880 (Terblanche, 1979). Angora goat numbers increased dramatically and by 1912 there were about 4,4 million goats in South Africa (Terblanche, 1979). The depression, the Second World War, droughts and a shortage of organization in the industry, plus the fact that soil erosion in certain areas has been attributed to Angora goats, caused a drastic reduction in Angora numbers (Terblanche, 1979). By 1939 there were only about 700 000 Angora goats left in South Africa (Terblanche, 1979). Today the Angora numbers have increased to well over a million, but will probably never again reach the heady hights attained

during 1912 (Terblanche, 1979). Today South Africa and the United States of America are the major mohair-producing countries in the world (Fourie *et al.*, 1995).

Damalinia limbata is an obligate ectoparasite of Angora goats. Records of these lice in South Africa date from the early part of the twentieth century when Bedford (1919) reported finding *D. limbata* on Angora goats at Onderstepoort in Gauteng (previously part of the Transvaal Province) and at Pietermaritzburg in Natal. But this does not, however, mean that these lice were introduced into South Africa at that time. The exact time of introduction is not known. *Damalinia limbata* was probably introduced into the country at the same time as the host.

Damalinia limbata was first described by Gervias in 1847 (Bedford, 1919). Except for taxonomic work done by workers such as Bedford (1919), Ewing (1936), Werneck (1936) and Lyal (1985b) very little is known about the biology and ecology of these lice. Hopkins & Chamberlain (1969) did some work on the *in vitro* rearing of *D. limbata* and found that the generation period for *D. limbata* was 32.2 days. The lice also had a preferred temperature of 35 ± 1.5 °C and a relative humidity (RH) of 76 % (Hopkins & Chamberlain, 1969). The authors also found that adult *D. limbata* laid an average of 0.8 eggs/day or roughly 2 eggs/3 days (Hopkins & Chamberlain, 1969). Fourie *et al.* (1995) did some work on the chemical control of these lice. No other references on the biology and ecology of these lice were found in literature surveys.

A great deal of work has, however, been done on a closely related species, *D. ovis* (Schrank), which is found on sheep. This species was used for comparison because of the similarity of environment and life cycle. Both species live on longhaired animals where the microclimate is reasonably stable. Hopkins (1970) used the same techniques to rear *D. ovis in vitro* as Hopkins & Chamberlain (1969) used, to rear *D limbata* and *D. crassipes*.

Scott (1952) studied the life cycle of *D. ovis* on sheep, and found that 36.5 °C was the optimum temperature for these lice. She also determined that the generation time of these lice was about 34 days. Scott (1952) also found that *D. ovis* had three nymphal stages (after hatching), followed by the adult male and female stages. Murray (1957 a-c) described and analyzed the oviposition behaviour and distribution of the eggs of *D. ovis*. It was observed that, about 1 hr before the *D. ovis* was ready for oviposition, the female moved to the warm end of the temperature gradient, where she remained until the egg was oviposited (Murray, 1957a). The lice were found to attach their eggs to the wool fibers of the sheep close to the skin and most eggs were deposited where the temperature was approximately 37.5 °C (Murray, 1957b).

The effects of humidity, rainfall and temperature on the population dynamics of *D. ovis* have also been quantified (Murray, 1960a; 1963; 1968 and Murray & Gordon, 1969). Murray (1960a) found that, *D. ovis* eggs, completed their development and hatched at temperatures ranging between 30-39 °C at RH of 7-75%. Murray (1963) found that immersion of eggs in water followed by exposure to high RH led to high mortality rates of all stages of the lice. Murray (1968) also found that exposure to high rates of solar radiation had a substantial influence on the mortality of *D. ovis*, especially after shearing.

Other research workers have studied and quantified the effects of *D. ovis* on wool quality and colour (Zumpt, 1970; Kettle & Lukies, 1982a; b; Wilkinson, *et al.*, 1982; Niven & Pritchard, 1985 and Cleland, Dobson & Meade, 1989). Kettle & Lukies (1982a) found that 6 out of 7 fleeces from lice infested sheep were less bright than their lice free counterparts. Murray & Edwards (1987) and Sinclair *et al.* (1989) studied the composition of an *in vitro* feeding medium. Studies have also been done on the control and management of *D. ovis* on sheep (Brightling, 1989; James *et al*, 1993; Rugg & Thompson, 1993 and Rugg *et al.*, 1995).

Except for the research of Hopkins & Chamberlain (1969) and Fourie *et al.* (1995) very little, if any, work has been done with regard to *D. limbata* on Angora goats. In the past, lice have not been perceived as important or serious pests on livestock. During the latter half of the twentieth century, however, work done on *D. ovis* (Scott, 1952; Zumpt, 1970; Kettle & Lukies, 1982a; b; Wilkinson *et al.*, 1982; Niven & Pritchard, 1985 and Cleland *et al.*, 1989) and some unpublished work on *D. limbata*, has shown these lice to have significant effects on the production and quality of wool and mohair. Mohair producers in South Africa are being hampered in their management of *D. limbata* on Angora goats, due to a lack of information on the biology and behaviour of these insects.

This study was done to answer some of the questions pertaining to the biology and distribution of *D. limbata* and to promote more effective and economical management of these lice on commercial farms. The following were investigated: (1) Aspects of the morphology of *D. limbata* and *D. ovis* were compared to demonstrate their basic differences. (2) Temperature studies were done to establish the environmental temperatures prevalent on the body of Angora goats, for use in the establishment of an *in vitro* colony of *D. limbata*. (3) Morphometric characters were used to determine the number of nymphal instars of *D. limbata*. (4) Seasonal changes in the populations of *D. limbata* and the environmental factors responsible were investigated. (5) The influence of *D. limbata* on the body mass of Angora goats and the production and quality of mohair, as well as the efficacy of different methods of control, were examined.

CHAPTER 2

COMPARATIVE MORPHOLOGICAL ASPECTS OF THE BITING LICE *DAMALINIA LIMBATA* AND *D. OVIS*.

2.1. Introduction.

Biting lice are well-known ecto-parasites of vertebrates. The sheep louse, *Damalinia ovis*, is a pest of sheep in Australia and various other countries (Clarke, 1990), including South Africa (Zumpt, 1970). The biting louse *D. limbata*, however, is a pest on Angora goats in South Africa (Fourie *et al.*, 1995) and in the United States of America (Hopkins & Chamberlain, 1969) and probably in most countries where these goats are found. The bionomics of *D. ovis* has been studied by Scott (1952). Aspects of the behaviour of these insects have been studied by Murray (1960a b, 1963, 1968) and Murray & Gordon (1969). Very little has been published on the morphology and physiology of either of these lice species. Clarke (1990) described the external morphology of the antenna of *D. ovis*, and Soler Cruz & Martin Mateo (1996) compared the so-called pit organs of several species of *Damalinia* (*Bovicola*), including that of *D. ovis*.

In this chapter, aspects of the morphology of these lice were compared in an effort to demonstrate their basic differences.

2.2. Material and methods.

Preparation of specimens for light microscopy consisted of washing them for 10 minutes in distilled water. This was done to remove all debris and dirt from the specimens, so that all the structures of the body would be clearly visible. The lice were then preserved by submersion in 70 % ethanol for 48 hours. The lice were mounted on slides in glycerol for the morphometrical measurements. The lice (*D.*

ovis and *D. limbata*) were studied under a Zeiss compound microscope at ×40 magnification for the antennal measurements and at ×7.5 magnification for the body measurements. Measurements of the lice were taken with a calibrated ocular mounted on the microscope. Twenty lice of each sex and of each species were used to measure the width and length of the head, thorax and abdomen. Each segment of both antennae of each individual was measured along its length. The body outlines were sketched with the aid of a drawing tube attached to the microscopes.

Two techniques were used to prepare the lice for scanning electron microscopy (SEM):

(1) Live *Damalinia limbata* were placed in a porous container with an approximate volume of $5cm^3$. The porous container was wetted with distilled water. The water-saturated container containing the lice was then submerged in liquid nitrogen for 3-4 minutes. The frozen container was placed in a glass beaker and placed in a freeze-drier at –50 °C for 48-72 hrs. The freezing process is necessary to prevent distortion of the body during the dehydration procedure. When the lice were completely dehydrated, they were mounted on a SEM stub. The mounted objects were placed in a sputter-coater for two minutes coating them with a thin layer of gold.

(2) Sheep body lice (*D. ovis*) were preserved in 70 % ethanol. The dehydration of these lice could, therefore, not be done in the same manner as with the live *D. limbata*. The specimens in the 70 % ethanol were chilled to 4°C. They were then removed and placed in 80 % ethanol at 4°C for 10 minutes. The specimens were then successively placed in 90%, 96% and absolute concentrations of ethanol for a period of 10 minutes in each concentration, at room temperature. The lice were then placed in a critical point drier. After drying, the lice were mounted on SEM stubs and coated with a thin layer of gold in a sputter-coater for two minutes.

2.3. Results and discussion.

2.3.1. *Damalinia limbata*

2.3.1.1. General.

Males and females of *D. limbata* differ significantly in size ($p<0.001$). Female lice of *D. limbata* had an average body length of 1.611mm (±0.031mm), while male lice were on average 1.371mm (±0.072mm) long (Table 2.1.). Werneck (1936) found females *of D. limbata* to be on average 1.85 mm long, which is substantially longer than the specimens used in this study. The males studied by Werneck (1936) were 1.42 mm long and are well within the range of the specimens used in the current study. *D. limbata* is small and robust in appearance, with a large number of seta on the head and antenna of the female. The male has fewer of these seta. The thorax is short and sturdy with three pairs of claw-like legs, one pair per segment.

Table 2.1. The body dimensions of male and female *Damalinia limbata*.

n=20	Length (mm)				Widths (mm)			
	Avg.	Min	Max	SD	Avg..	Min	Max	SD
Male								
Head	0.301	0.253	0.336	±0.023	0.353	0.289	0.395	±0.027
Thorax	0.166	0.140	0.195	±0.016	0.273	0.233	0.320	±0.027
Abdomen	0.904	0.803	1.003	±0.054	0.638	0.566	0.693	±0.039
Tot. Body	1.371	1.196	1.534	±0.072	-	-	-	-
Female								
Head	0.355	0.317	0.407	±0.026	0.419	0.374	0.478	±0.030
Thorax	0.199	0.165	0.236	±0.020	0.327	0.275	0.378	±0.032
Abdomen	1.057	0.975	1.111	±0.035	0.745	0.688	0.816	±0.033
Tot. Body	1.611	1.457	1.754	±0.031	-	-	-	-

2.3.1.2. Head.

Damalinia limbata has head dimensions that are polygonal, the width being greater than the length for both sexes (Table 2.1). The head of the male is on average ×1.173 wider than the length; while the female head is on average ×1.180 wider than the length. This is in agreement with Werneck (1936), who stated that the head width is greater than the length. Female lice has a head length on average 15.2% and a width on average 15.8% greater than those of males

The anterior margin of the head is well sclerotised. The sclerotised anterior margin of the head is wide in *D. limbata* females. The anterior margin of the head, or forehead, is slightly concave in both sexes of *D. limbata* (Figs. 2.1 & 2.2.). The anterior margin of the head of the male is slightly less concave than that of the female. According to Ledger (1980), concave anterior margins are found in the more aberrant members of this genus, where the norm is a convex forehead. There is a large number of setae along the anterior margin of the head of the female. The anterior margin of the head of the male lice is covered with fewer setae. These lice have tentoria, on the dorsal face of the head, which converges from the anterio-lateral margins towards the middle of the head. Here they change direction and run parallel to each other until they reach the posterior margin (Figs. 2.1A & 2.2A). These tentoria are prominent (large) in *D. limbata*. The eyes of this species were situated temporally and are not prominent.

The trabeculae, which precede the antennae on the antrio-lateral sides of the head, were pronounced in *D. limbata*, obscuring almost half of the first antennal segment (Figs. 2.1 & 2.2). *D. limbata* possess large *labral membranis foramina* on the ventral face of the head. The *labral membranis foramen* has an almost elliptical shape in *D. limbata* females and triangular in males (Figs. 2.1B & 2.2B).

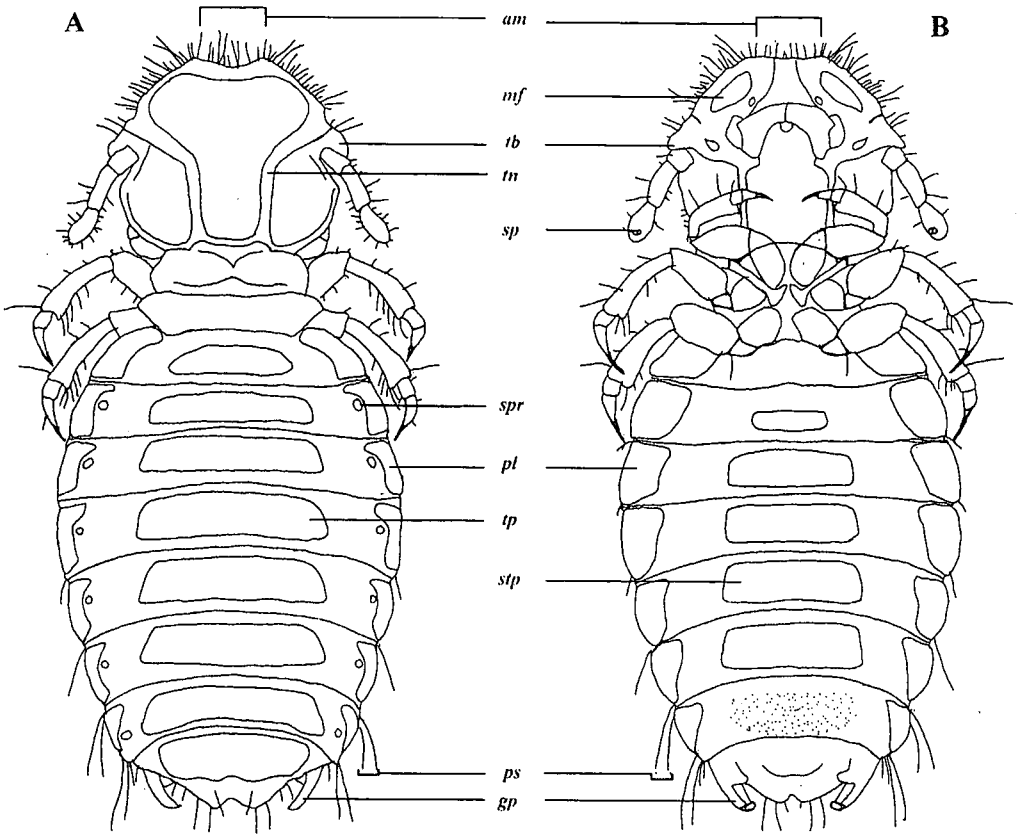

Fig. 2.1. Female *Damalinia limbata* with (A) the dorsal and (B) the ventral aspects. Key to the abbreviations: *am* = anterior margin; *gp* = gonopoda; *mf* = labral membranous foramen; *pl* = pleurae; *ps* = pleural seta; *sp* = sensory pits; *spr* = spiraculum; *stp* = sternal plate; *tb* = trabeculae; *tn* = tentorium; *tp* = tergal plate.

Table 2.2. Antennal measurements of male and female *Damalinia limbata*.

n=40	Avg.	Min	Max	SD
Male				
Seg1	0.015	0.013	0.018	0.001
Seg2	0.024	0.022	0.026	0.001
Seg3	0.023	0.019	0.026	0.001
Tot. Ant.	0.062	0.054	0.070	0.004
Female				
Seg1	0.014	0.012	0.015	0.001
Seg2	0.024	0.021	0.026	0.001
Seg3	0.027	0.025	0.029	0.001
Tot. Ant.	0.065	0.058	0.071	0.003

An analysis of variance showed that males of *D. limbata* have significantly shorter antennae than the females ($p<0.001$). *D. limbata* males had antennae with the second and third antennal segments equal in length, but almost 1.5X as long as the first segment (Table 2.2.). The females showed similar features. The total length of male *D. limbata* antennae are on average 5 % shorter than those of the female.

2.3.1.3. Thorax.

The thorax of both males and females is short and wide, with the width being about four-tenths greater than the length (Table 2.1). Only the meso- and metathorax are dorsally visible in both sexes (Figs. 2.1A & 2.2A). The metathorax of both sexes is concave on the posterior margin. In *D. limbata* the lateral margins of all segments are strongly sclerotised and prominent. The legs of the prothorax are shorter compared to those on the meso- and metathorax, which in turn are about the same size. This is in accordance with the data reported by Werneck (1936) who also noted that the legs of this species are characteristic of the genus. The legs are adapted for grasping and moving along the hair of the host. Werneck (1936) further stated that the morphometrics and morphology of the legs are not of great use for taxonomic purposes.

2.3.1.4. Abdomen.

The abdomen of *D. limbata* is wide and oval and has an almost squat appearance (Fig. 2.1) but is more pointed posteriorly in the males (Fig. 2.2). In both sexes, the abdomen forms the longest and widest part of the body. The length of the abdomen represents about 66 % of the total body length in both sexes (Table 2.1). In both sexes, the length of the abdomen equals about 1.4 times the width. The abdomina of male lice are, however, generally shorter and narrower compared to those of the females (Table 2.1).

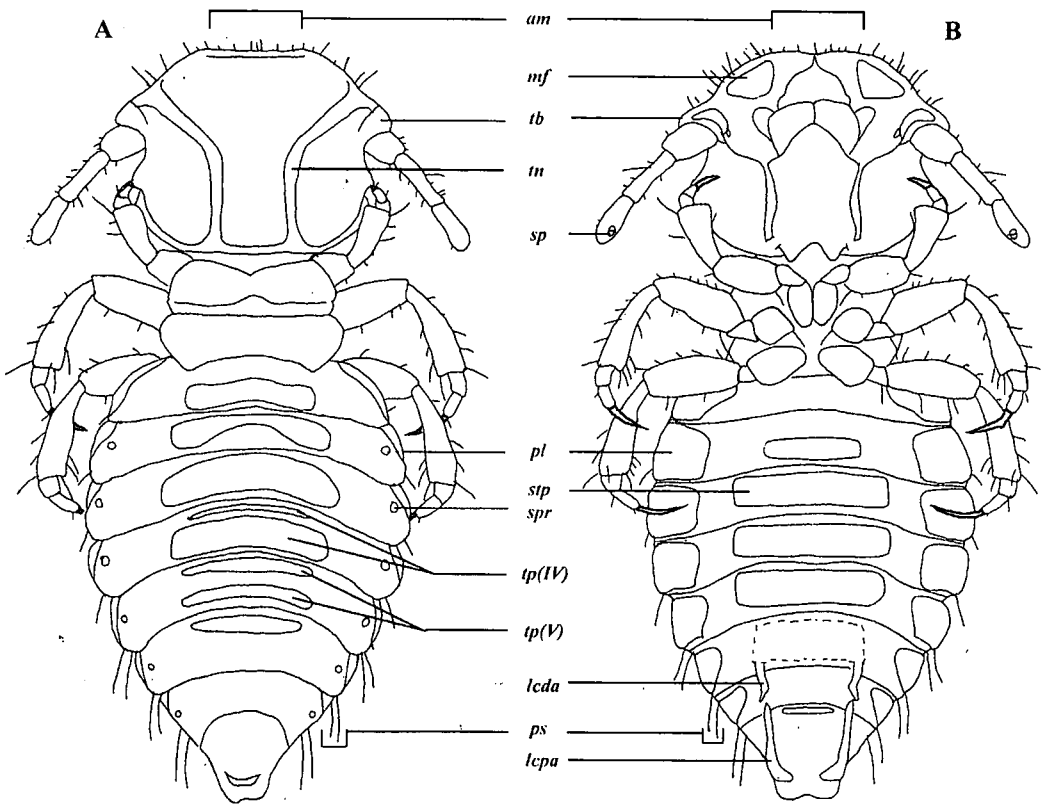

Fig. 2.2. Male *Damalinia limbata* with (A) the dorsal and (B) the ventral aspects. Key to the abbreviations: *am* = anterior margin; *lcda* = lateral caudal apophysis; *lcpa* = lateral cephalic apophysis; *mf* = labral membranous foramen; *pl* = pleurae; *ps* = pleural seta; *sp* sensory pits; *spr* = spiracle; *stp* = sternal plate; *tb* = trabeculae; *tn* = tentorium; *tp(IV)* = tergal plates of segment IV; *tp(V)* = tergal plates of segment V.

The dorsal face of the female abdomen is characterized by an arrangement of well-sclerotised tergal plates, which are all transversally elongated (Figs 2.1A). The female possess eight of these tergal plates dorsally (found on segments I – VIII) and ventrally possesses five sternal plates (found on segments II – VI), which were also transversally elongated (Fig. 2.1B). In contrast, the males possesses only six tergal plates dorsally (found on segments I – VI). The tergal plates of the males are transversally split on segments IV and V (Fig. 2.2A). The anterior plate of segment IV is narrower than

the posterior plate. The tergal plate on segment V is subdivided into two similar sized plates. In males the sterna on segment VI are poorly defined (almost not discernable), but there are two lateral caudal apophyses, extending posteriorly, but only to segment VII (Fig. 2.2B). Two lateral cephalic apophyses of sternites VIII and IX extend anteriorly to segment VII, where they end in close proximity to the posterior tip of the lateral caudal apophyses (Fig. 2.2B).

In both sexes the dorsal face of the abdomen possesses six pairs of spiracles, which are upward facing, and are situated laterally, close to the sclerotised pleura of segments II – VII (Figs. 2.1A & 2.2A). The pleural size of both sexes decreases from the anterior to the posterior of the abdomen. The pleura of *D. limbata* are large and clearly defined. The setae arising ventrally from the posterior side of each pleura is long. *D. limbata* females possess two pairs of these seta on each of segments VI and VII (Fig 2.1A & B), whereas the males have them on segments V, VI and VII (Fig 2.2A & B). The external genitalia of the female are situated laterally on the posterior end of the abdomen originating from segment VIII. The gonopods are well defined and prominent (Fig. 2.1A & B). The genitalia of the males are situated internally on the posterior end of the abdomen.

2.3.2. *Damalinia ovis.*

2.3.2.1. General.

Female lice of *D. ovis* have an average length of 1.612 mm and the males on average 1.253 mm long (Table 2.3). This is shorter than recordings by Werneck (1936), who found the females to be on average 1.77 mm and the males to be on average 1.55 mm long. *D. ovis* females and males are slender and have a more or less streamlined appearance. An analysis of variance showed the females to be significantly longer than the males ($p < 0.001$).

Table 2.3. The body dimensions of male and female *Damalinia ovis*.

n=20	Length (mm)				Widths (mm)			
	Avg.	Min	Max	SD	Avg.	Min	Max	SD
Male								
Head	0.315	0.288	0.333	±0.012	0.311	0.284	0.328	±0.011
Thorax	0.150	0.132	0.172	±0.010	0.232	0.206	0.270	±0.017
Abdomen	0.788	0.737	0.909	±0.040	0.447	0.415	0.511	±0.021
Tot. Body	1.253	1.157	1.414	±0.024	-	-	-	-
Female								
Head	0.355	0.320	0.402	±0.017	0.360	0.326	0.407	±0.018
Thorax	0.254	0.230	0.274	±0.012	0.315	0.283	0.341	±0.016
Abdomen	1.003	0.924	1.082	±0.043	0.606	0.562	0.640	±0.026
Tot. Body	1.612	1.474	1.758	±0.035	-	-	-	-

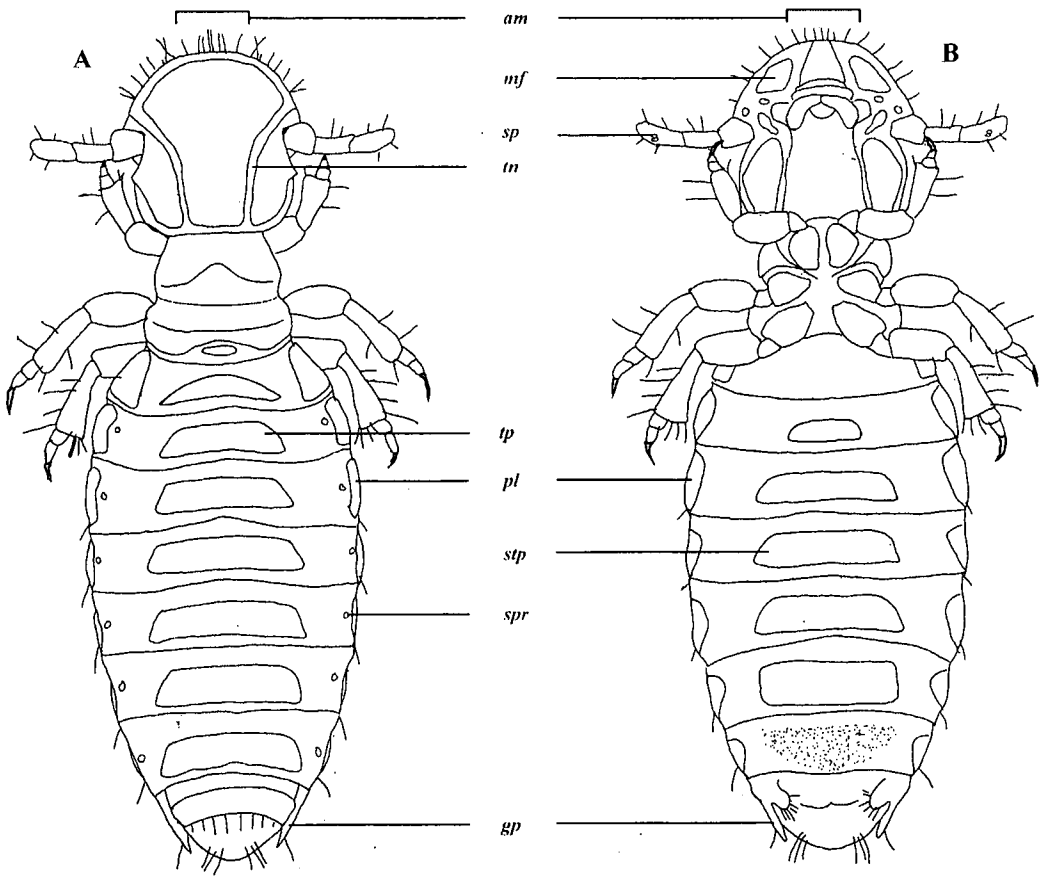

Fig. 2.3. Female *Damalinia ovis* with (A) the dorsal and (B) the ventral aspects. Key to the abbreviations: *am* = anterior margin; *gp* = gonopods; *mf* = labral membranous foramen; *pl* = pleurae; *sp* = sensory pits; *spr* = spiracle;. *stp* = sternal plate *tn* = tentorium; *tp* = tergal plate;

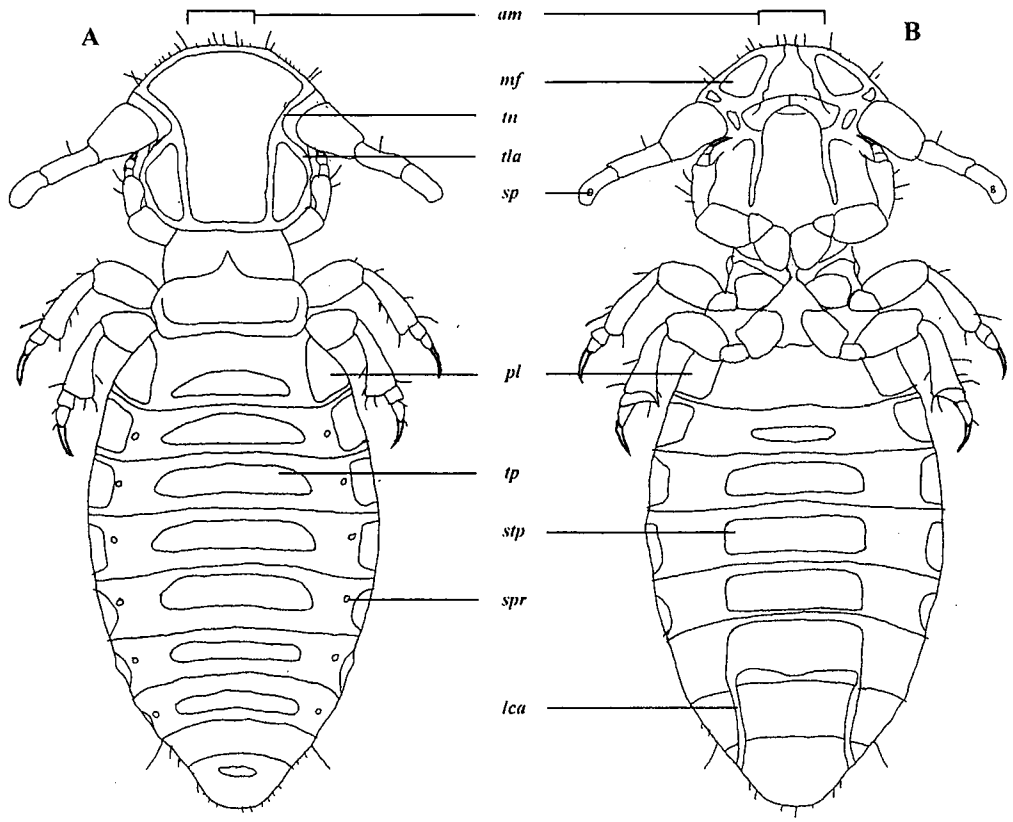

Fig. 2.4. Male *Damalinia limbata* with (A) the dorsal and (B) the ventral aspects. Key to the abbreviations: *am* = anterior margin; *lca* = lateral caudal apophysis; *mf* = labral membranous foramen; *pl* = pleurae; *sp* = sensory pits; *spr* = spiracle; *stp* = sternal plate; *tla* = tentorial arm; *tn* = tentorium; *tp* = tergal plate.

2.3.2.2. Head.

The head of *D. ovis* is circular in form with the length being about equal to the width for the males as well as for the females (Table 2.3). The anterior margin is well sclerotised in both sexes. The anterior margin of the head is convex in both sexes, which is typical of this genus (Ledger, 1980). *D. ovis* males have a less convex form on the anterior margin of the head than the females (Fig. 2.3A, B & 2.4A, B). *D. ovis* has only a few setae along the anterior margin of the head.

Both sexes have tentoria, on the dorsal face of the head, which converge from the anterio-lateral margins to the middle of the head, where they change direction and run parallel to each other until they reach the posterior margin (Fig. 2.3A & 2.4A). *D. ovis* males possess a distal tentorial arm (*tla*), which branches off from the main tentoria a third of the way from the anterior margin from where it changes direction (Fig. 2.4A). This tentorial arm ends at the lateral margin of the head, posterior of the antennal attachment (Fig. 2.4A).

The trabeculae, which precede the antennae on the antrio-lateral sides of the head, are not very pronounced in *D. ovis,* and are in fact almost indiscernible. Both sexes possess large *labral membranis foramina* on the ventral face of the head. The *labral membranis foramina* have a triangular shape in both sexes of *D. ovis*.

Table 2.4. Antenna measurements of male and female *Damalinia ovis*.

	Avg.	Min	Max	SD
Male				
Seg1	0.027	0.020	0.035	±0.003
Seg2	0.025	0.022	0.028	±0.002
Seg3	0.024	0.020	0.027	±0.002
Tot. Ant.	0.076	0.069	0.083	±0.004
Female				
Seg1	0.017	0.013	0.022	±0.002
Seg2	0.027	0.020	0.033	±0.003
Seg3	0.025	0.022	0.027	±0.001
Tot. Ant.	0.069	0.063	0.079	±0.004

D. ovis females had antenna with the second and third segments about equal in length and the first segment was about two-thirds the individual length of the other two (Table 2.4.). The antennal segments of *D. ovis* males, however, were all about the same length (Table 2.4). The antennae of males were on average one-tenth shorter than the antennae of females. The first antennal segments of males are also much broader than the females (Fig. 2.3A,B and 2.4A,B).

2.3.2.3. Thorax.

The thorax of *D. ovis* has a width about ×1.4 greater than the length (Table 2.3.). The metathorax of both sexes of *D. ovis* is concave on the posterior margin. Both sexes possess legs characteristic of this genus, which are adapted to hold on to the hair of the host.

2.3.2.4. Abdomen.

D. ovis has a long and oval abdomen, with a slender and streamlined aspect in both sexes. The abdomen is longer than wide for both sexes (Table 2.3.). The length of the abdomen represents about 63% of the total body length of this lice species.

The dorsal face of *D. ovis* is characterized by an arrangement of well-sclerotised tergal plates on the abdomen, which are transversally elongated (Fig. 2.3A and 2.3B). The female of this species possess eight of these tergal plates, which are found on segments I – VIII (Fig. 2.3A) and the males possess seven of these tergal plates which are found on segments I – VII (Fig. 2.4A). Ventrally both sexes possess five sternal plates, which are also transversally elongated (Fig. 2.3B and 2.4B). In both species, the sternal plates were found on segments II – VI. The sternite of segment VI has a pair of lateral caudal apophyses, which extends posteriorly to segment IX of the male *D. ovis* (Fig. 2.4B).

In both sexes the dorsal face of the abdomen possess six pairs of spiraculae, which are upward facing, and are situated laterally, close to the sclerotised pleura of segments II – VII (Fig. 2.3A and 2.4A). The pleurae of both sexes decrease in size, from the anterior to the posterior of the abdomen. The seta arising ventrally from the posterior side of each pleura, are relatively short in *D. ovis*. The external genitalia of the female are situated laterally on the posterior end of the abdomen originating from segment VIII (Fig. 2.3A,B). The pair of

gonopods is well defined. The genitalia of the male are situated internally on the posterior end of the abdomen.

2.3.3. *Damalinia limbata* compared to *Damalinia ovis*.

2.3.3.1. Head.

The basic differences between the two species are obvious. The body of *D. limbata* is much more robust in appearance than *D. ovis*, which appears slender and streamlined (Figs. 2.1; 2.2; 2.3 & 2.4).

The anterior margin of the head of *D. ovis* is convex, while the anterior margin of the head of *D. limbata* is concave (Figs. 2.1; 2.2; 2.3 & 2.4). *D. limbata* has prominent trabeculae, which obscures a large portion of the first antennal segment anterio-laterally. This is not so in *D. ovis*, where the trabeculae are small and non-obtrusive (Figs. 2.1A,B; 2.2A,B; 2.3A,B & 2.4A.B). Male *D. ovis* has lateral tentorial arms on the dorsal face of the head, which are absent in males of *D. limbata* (Figs. 2.2A, & 2.4A). *D. ovis* has a head length slightly longer in males and equal in the female compared to those of *D. limbata*. In contrast, both sexes of *D. limbata* have head widths which are greater than those of *D. ovis* (Tables 2.1 & 2.3).

Both sexes of *D. limbata* appeared to have longer antenna than *D. ovis* (Table 2.2. & 2.4.). *D. limbata* males and females have antennae of which the second and third antennal segments are equal in length, but are about twice as long as the first segment (Table 2.2). The females of *D. ovis* also displayed this trend but the males, however, have antennae of which the segments are of similar length (Table 2.4). The antennal length of *D. limbata* is on average shorter than the antenna of *D. ovis* (Tables 2.2 & 2.4).

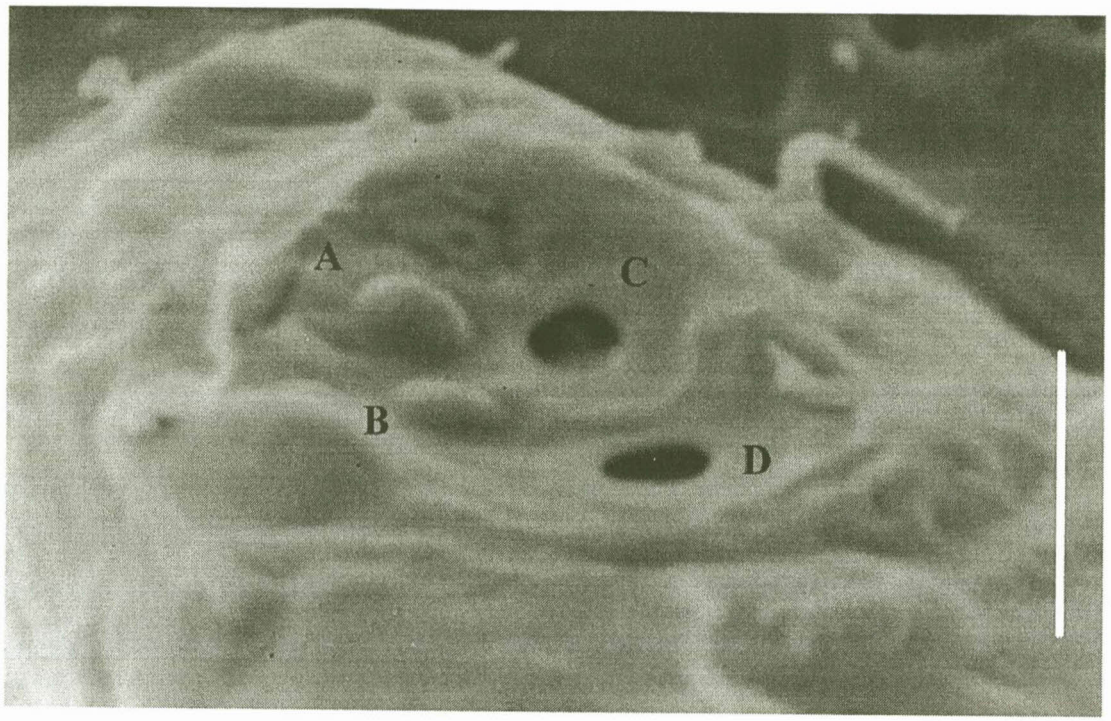

Fig. 2.5. Detail of pits on antennal segment 3 of *Damalinia limbata*. Scale bar = 10 μm.

Clarke (1990) found pits on the third antennal segment of *D. ovis*. Sensilla were present in the pits, which he called "pit-organs". He proposed that these sensilla might be present throughout the family.

Soler-Cruz & Martin-Mateo (1996) compared these pits and pit-organs on the antenna of *D. ovis* with *D. carprae*, *D. bovis* and *D. breviceps*, respectively, and confirmed their presence in all except *D. breviceps*.

Scanning electron microscopic photographs of the third antennal segments of *D. ovis* and *D. limbata* (Figs. 2.5. & 2.6.) were taken and it was found that *D. limbata* also possessed these pits with the pit-organs in them. Unfortunately, these pit-organs could not be clearly photographed. These results would seem to confirm the theory of Clarke (1990). This is also supported by Soler-Cruz & Martin-Mateo (1996) who found these structures in all the species examined except in *D. breviceps*.

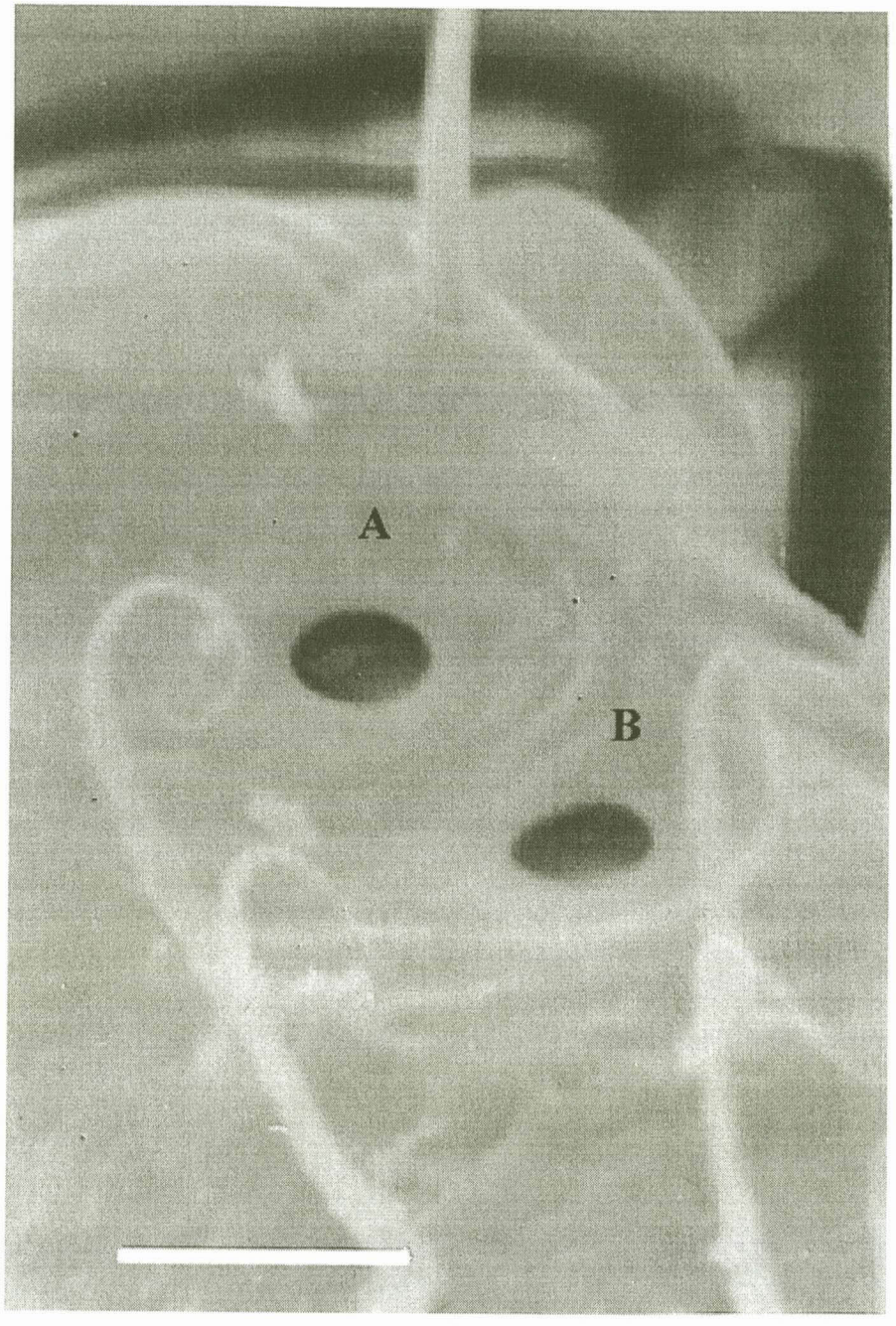

Fig. 2.6. Detail of pits on antennal segment 3 *of* *Damalinia ovis*. Scale bar = 10 μm.

There are differences between the sensory pits of *D. limbata* and *D. ovis*. The concave area around the pits (structures A & B in Fig. 2.6.) of *D. ovis* is smooth, without visible protrusions. The concave area around the pits (structures C & D in Fig. 2.5.) is not smooth in *D. limbata*, but is characterized by two bullae (structures A & B in Fig. 2.5.). These bullae are situated to the anterior of pit C (Fig. 2.5).

2.3.3.2. Thorax.

Both species have thoracic dimensions where the width is greater than the length. This contradicts the findings of Werneck (1936), who found that in *D. ovis* the thorax length exceeds its width. Werneck (1936) did find, however, that *D. limbata* has a greater thorax width than length. In the case of females, *D. ovis* has a greater average thorax length. The males, however, displayed an inverse trend (Tables 2.2 & 2.4). In *D. limbata,* both sexes have a greater thorax width than *D. ovis*.

2.3.3.3. Abdomen.

Both sexes of *D. limbata* have abdomen lengths and widths greater than that of *D. ovis* (Tables 2.2 & 2.3). The most apparent differences between the two species are found in the males. The dorsal face of *D. limbata* has the tergal plates of segments IV and V transversally split, whereas *D. ovis* has tergal plates which are solid on these segments (Figs. 2.2A & 2.4A). The ventral face of *D. ovis* males has two lateral caudal apophyses, which extend from the sternal plate of segment VI to segment IX (Fig. 2.4B). Sternal plate VI is poorly defined in *D. limbata* males and the lateral caudal apophyses only extend to segment VII (Fig. 2.2B). In addition, *D. limbata* has two lateral cephalic apophyses, which extend anteriorly from segment IX to segment VII (Fig. 2.2B).

These are the most important morphological differences between the two species.

CHAPTER 3

TEMPERATURE STUDIES AND THE IN VITRO COLONIZATION OF *DAMALINIA LIMBATA*.

3.1. Introduction.

During biological and bionomic studies of Mallophaga, researchers have been able to maintain several species *in vitro* for short periods (Scott, 1952). Some of these researchers have even been able to sustain *in vitro* colonies for prolonged periods of time (Murray, 1963; Hopkins & Chamberlain, 1969). However, successes in this regard seem to be infrequent. The difficulty, in maintaining *in vitro* colonies for extended periods, can be attributed to the specialized feeding habits and food sources of these lice (Murray, 1963).

Biting lice such as *Damalinia limbata* and *D. crassipes*, on goats, have been reared *in vitro* successfully by Hopkins and Chamberlain (1969). This kind of work is important to obtain material such as eggs and nymphs with which to conduct experiments pertaining to the general biology and ecology of *D. limbata*.

The purpose of this study was firstly to ascertain the environmental temperatures to which *D. limbata* are exposed to on Angora goats and secondly attempts were made to rear *D. limbata in vitro*. The data generated from the temperature studies were used in an attempt to rear the lice *in vitro*. The laboratory colony would be used to produce eggs, nymphs and adults for further biological and ecological studies.

3.2. Material and methods.

3.2.1 Temperature variation in the micro-habitat of *Damalinia limbata*

An Angora goat was taken from the heard and confined in a pen for a 20 hour period. During the period of study the goat was continuously exposed to the sun during the day. The skin temperature (this was taken to be the temperature against the skin of the goat) and hair-tip temperatures were monitored at three different areas on the body of the goat. These areas were the back (dorsal), one flank (lateral) and the abdomen (ventral). The two temperature readings on each area (to the nearest 0.1 °C) were taken at four hourly intervals using two thermocouples connected to a data-logger. The temperature readings were recorded at 12:00; 16:00; 20:00; 00:00; 04:00 and 08:00 respectively. These studies were repeated three times, in July and October 1994 and again in January 1995. During the summer and winter (January and July) the mohair length, of the Angora goats, was medium to long (8-12 cm). The mohair length during spring (October) was shorter (5-6 cm), because the goats were shorn in late July.

3.2.2. Rearing a laboratory colony of *Damalinia limbata*.

The techniques used were based on those of Hopkins & Chamberlain (1969). Food was prepared by cutting fresh, closely sheared Angora goatskin into 20 X 20 cm sections. These sections were placed flesh side down on glass plates and frozen at –20 °C. Forty-eight hours after freezing, a sharp blade was used to scrape off the flaky outer portion of the frozen skin. The frozen skin plates were kept at –20°C, for further use later. The scrapings were then dried for 24 hrs at 35 °C. Using a stainless steel liquidizer, the scrapings were cut into particles small enough to pass through a No. 25 mesh sieve. The material obtained was placed in moisture proof containers at –20°C.

The lice used to start the *in vitro* colonies were obtained from Angora goat mohair clippings infested with *D. limbata*. In separating the lice from the hair a Berlese funnel with a 25cm diameter glass funnel was used to extract the lice. An infrared lamp was placed above some loosely packed, louse containing mohair in the funnel. The lamp was moved closer to the mohair every 15-20 minutes driving the lice from the mohair. Since the lice can not cling to a glass surface, all lice falling onto the side of the funnel slid down and fell into a glass beaker placed underneath it. The nymphs and adults were separated. The nymphs were discarded and the adults placed in glass vials (90mm X 25mm Poly-top vials) at 50-200 lice per vial (as described by Hopkins & Chamberlain, 1969). The adults were maintained at a ratio of 1 male: 3 females. Skin-scrapings were placed in the vials at a rate of 175 mg/vial and the unutilized food replaced every two weeks. The vials were placed in 50ml glass beakers which, in turn were placed in polystyrene containers with tight-fitting lids, large enough to accommodate 5-6 beakers. A platform with holes was placed in each of the containers on which the beakers were placed.

The required relative humidity of 76 % was maintained by pouring 100 ml of a saturated, aqueous solution of NH_4Cl in the polystyrene containers. The temperature was regulated by keeping the containers, at 35 ± 3°C (calculated from field temperature data as well as the results of Hopkins & Chamberlain, 1969), in an electronically controlled temperature cabinet. The lice were fed, handled and studied, outside the controlled environment but care was taken not to expose the eggs to temperatures below 27 °C. The photoperiodic cycle consisted of 15 hr of dark and 9hr dim light daily (Hopkins & Chamberlain, 1969).

D. limbata cement their eggs to mohair when they are on a host. A number of strands (20-30 strands), of unwashed goat hairs, 6-8 cm in length, were made into loose coils and placed in the vials for oviposition. The lice and food were then added. It was also ensured that the bottom portions of the mohair coils were covered with food because, according to Hopkins & Chamberlain (1969), these lice seem

to prefer ovipositing on the part of the hair strand situated below the surface of the food. On the host, the lice seem to prefer oviposition sites close to the skin, where the eggs are in close proximity to loose skin scruff and other skin debris. Observations were made every 3-4 days and mohair strands with eggs attached to them, were removed and placed in vials for incubation, at a rate of 100 eggs per vial. Loose eggs, found in the food were also removed and placed in separate vials at the same rate.

During the second and third attempts at establishing colonies (started on 16/2/1995 and 1/3/1995 respectively), variations in yeast concentrations were tried in the diet of the lice. The RH and the temperature were kept constant at 76% and 35 ± 1°C respectively. Brewers-yeast was finely ground and added to the skin scrapings at a weight ratio of 1:1, 1:2 and 1:3 yeast: food for the second colonization attempt. The colony only lasted 14 days with four eggs collected from the 1:3 yeast/food mixture, of which none hatched. The yeast concentration might have been too high and the yeast to food ratio was adjusted to the following: 1:8, 1:10 and 1:15, for the third colonization attempt. For this attempt, the RH and temperature were also kept constant at 76% and 35 ± 1°C.

With the fourth attempt at establishing a colony (started 08/05/1995), *D. limbata* populations were kept at various relative humidities (RH) *viz.* 51, 66 and 76% RH using solutions of $NaCr_2O_4.H_2O$; KOH and NH_4Cl (Solomon, 1951; Winston & Bates, 1960). The temperatures (35°C) were the same for all the experimental groups.

3.3. Results and discussion.

3.3.1 Temperature variation in the micro-habitat of *Damalinia limbata*

It is evident that the skin temperature of Angora goats stays fairly constant throughout the day (Table 3.1.). During mid-winter, (July

1994) the average skin temperature (of the three areas combined) for the entire observation period, was 33.5°C. These average temperatures varied between 36.3 °C, at 12:00 and 31.5 °C, at 00:00 in the morning (Table 3.1).

Table 3.1. Data for the skin, hair-tip and environmental temperatures (°C) of Angora goats, for a 20hr period during July and October 1994 and January 1995.

	Dorsal		Lateral		Ventral		Average		Environ
	Skin	Tip	Skin	Tip	Skin	Tip	Skin	Tip	Temp.
Jul. 94									
12:00	34.4	43.5	37.4	26.6	37.2	24.7	36.3	31.6	17.2
16:00	32.3	20.6	36.5	21.3	31.5	20.7	33.4	20.9	15.2
20:00	31.5	15.0	33.4	18.3	32.0	11.0	32.3	14.8	5.6
00:00	31.2	10.3	32.1	11.2	31.3	10.5	31.5	10.7	1.9
04:00	32.1	8.3	31.2	9.9	32.3	6.2	31.9	8.1	-6.0
08:00	35.9	22.1	36.1	23.5	34.9	16.3	35.6	20.6	4.2
Avg.	32.9	20.0	34.5	18.5	33.2	14.9	33.5	17.8	6.4
Oct. 94									
12:00	35.8	51.8	30.5	40.6	36.6	37.5	34.3	43.3	29.6
16:00	38.4	50.0	35.7	29.0	36.2	28.5	36.8	35.8	25.1
20:00	32.5	18.0	35.1	20.2	32.1	19.5	33.2	19.2	13.1
00:00	31.5	14.3	33.2	18.2	33.2	15.1	32.6	15.9	8.9
04:00	29.3	14.1	30.9	16.5	30.2	14.7	30.1	15.1	3.9
08:00	28.1	16.5	31.9	17.2	25.6	16.1	28.5	16.6	15.1
Avg.	32.6	27.5	32.9	23.6	32.3	21.9	32.6	24.3	16.0
Jan. 95									
12:00	38.9	48.3	39.5	40.6	38.5	38.6	39.0	42.5	35.9
16:00	37.8	39.1	38.1	39.1	36.8	38.7	37.6	39.0	33.9
20:00	38.0	24.5	38.6	29.3	38.1	28.1	38.2	27.3	25.9
00:00	36.1	25.2	35.7	25.5	37.2	24.9	36.3	25.2	22.5
04:00	36.2	24.1	35.1	26.4	37.3	25.3	36.2	25.3	15.6
08:00	36.5	45.4	38.8	39.5	37.6	30.3	37.6	38.4	26.0
Avg.	37.3	34.4	37.6	33.4	37.6	31.0	37.5	32.9	26.6

Average skin temperature for the three months was 34.5 ± 3 °C.

The average mohair-tip temperature (for the three areas combined) for the study period, was 17.8 °C with an average maximum of 31.6 °C at 12:00 and a minimum of 8.1°C at 04:00 in the morning (Table 3.1). The highest individual temperature was recorded dorsally at noon (43.5°C)

and the minimum was recorded ventrally at 04:00 (6.2°C) (Table 3.1). At the same time, the environmental temperature also reached a minimum.

During October (1994) the average skin temperature (of the three areas combined) for the observation period was 32.6 °C, with an average maximum of 36.8°C at 16:00 and an average minimum of 28.5 at 08:00. The skin temperature of the individual areas reached a maximum of 38.4 °C dorsally at 16:00 (Table 3.1). The minimum individual skin temperature was 25.6°C recorded ventrally at 08:00 (Table 3.1).

The average temperature at the hair-tip (of the three areas combined) was 24.3 °C with an average maximum of 43.3 recorded at noon and an average minimum of 15.1°C recorded at 04:00. The maximum individual hair-tip temperature was 51.8°C, recorded at noon (Table 3.1). The minimum individual hair-tip temperature was 14.1°C recorded dorsally at 04:00 (Table 3.1).

In January 1995, the average skin temperature (of the three areas combined) for the observation period was 37.5 °C (Table 3.1). The average maximum skin temperature was 39°C, recorded at noon (Table 3.1). The maximum individual skin temperature was recorded laterally, at noon (39.5°C) (Table 3.1). The individual minimum temperature of the skin (35.1°C) was recorded at 04:00 in the morning on the ventral (abdominal) area of the goat (Table 3.1).

The average hair-tip temperatures (of the three areas combined) for the observation period was 32.9 °C (Table 3.1). The average maximum temperature at the hair-tip was 42.5°C recorded at noon (Table 3.1). The individual maximum for hair-tip temperature was recorded dorsally, also at noon (48.3°C). The individual minimum temperature of the hair-tips (24.1°C) was recorded at 04:00 in the morning and was recorded on the dorsal area of the goat (Table 3.1).

The highest average hair-tip temperatures were consistently recorded on the dorsal area of the Angora goat with individual temperatures usually reaching a maximum at around noon. This trend correlates well with the findings of Murray (1963), who did a temperature study on the body of sheep. He found that the highest temperatures were recorded on the back of the sheep, with progressively lower temperatures down the flanks and the lowest temperatures on the belly. An explanation for this phenomenon is that the back of the animal is the area most exposed to direct solar radiation and for the most prolonged periods (Murray, 1960a, 1963, 1968). In contrast to hair-tip temperatures, the average temperatures on the skin of the goats remained relatively constant with an average of 34.5 ± 3°C (range: 28.5 - 39°C) for the three dates recorded (Table 3.1). Murray (1963) found that the average skin temperature on sheep were 36.5 ± 1°C. This is higher than recorded on angoras but the study was also done in mid-summer (Murray, 1963). In mid-summer (January) the average skin temperature of angoras was 37.5 °C which is well within the range reported by Murray (1963).

During October, the degree of variation in the skin temperature was greater than during July and January (Table 3.1). This variation, however, was probably due to the shortness of the mohair, (the goats were shorn in late July) exposing the skin to greater environmental variation than during the other two months. These results would seem to support the findings of Hopkins & Chamberlain (1969) which states that 35 ± 1.5°C is the optimum temperature for rearing lice and was consequently accepted as such for use in rearing a laboratory colony.

3.3.2. Rearing a laboratory colony of *Damalinia limbata.*

Four attempts were made to establish *D. limbata* colonies *in vitro*. On the 8th of August 1994, a colony was started with 10 vials containing 200 adult lice each. The lice were kept in the conditions as specified by Hopkins & Chamberlain (1969) and inspected every 3-4 days. After 16 days 57 eggs had been collected from the initial population and incubated in separate vials. No eggs were found beyond 16 days and

more than 80% of the lice in the population died within 24 days. Only nine of the eggs collected hatched, four after 10 days, two after 11 days and three after 13 days. After 38 days, however, all lice in the population had died. During the second and third attempts, the colony lasted 21 days with 27 eggs collected after 12 days (1:8 yeast/food mixture (nine eggs); 1:10 yeast/food mixture (seven eggs) and 1:15 yeast/food mixture (11 eggs.)). However, by this time 87% of the population had died. Of the 27 eggs collected only three hatched, all from the 1:15 yeast/food mixture. None survived to the second instar.

The reason, for the failure of the colony, was possibly due to the absence or shortage of the natural flora of bacteria found on the body of the host. A bacterial supplement, in the form of yeast, was added to food of the lice. This approach of adding yeast to the diet of the lice was also used by Scott (1952) during the artificial rearing of *D. ovis*. A rich bacterial flora is present on the skin surface of sheep. Murray & Edwards (1987) found the species composition of bacteria on the skin of the sheep to be highly variable and susceptible to rapid change. Living bacteria and other living organisms were found to constitute a significant proportion of the diet of lice and can not be ignored as a potentially important source of amino acids, vitamins and other elements (Murray & Edwards 1987).

Murray (1960b) stated that, for *D. ovis* on sheep, the optimum conditions for oviposition was 54% RH and 37°C. This is in contrast with Hopkins & Chamberlain (1969) who found 76% RH to be the optimum for oviposition of *D. limbata*. Murray (1960a,b) found that the eggs of *D. ovis* could be prevented from hatching by high RH values.

An attempt was made to start a colony using activated charcoal mixed with gypsum (plaster of Paris) and poured into the individual vials. The mixture, when left for a while, hardened and provided a porous base to absorb water. This technique was not very successful, because the water in the vial caused the dried skin to become soggy and

unattractive to the lice. The interaction of the water with the yeast was also not conducive to the survival of the lice. The colony was kept in vials and provided with food as described by Hopkins & Chamberlain (1969). All three groups, however, died after 11 days and no eggs were found in any of the vials.

It was also suspected that the quality of the food used had degraded and was not fit for consumption by *D. limbata*. Hopkins & Chamberlain (1969) reported that the skin squares, mounted on glass, could be stored for prolonged periods if frozen. Even frozen goods, however, will degrade eventually. The goat skin used for this experiment had been prepared in 1993 and after about two years in the deep freeze the quality would probably have been substandard. Because of budgetary constraints, fresh skins, for use as food medium, could not be obtained.

3.4. Conclusion.

The temperature, in the micro-habitat of *D. limbata,* stayed relatively constant at approximately 35°C. However, a sustainable colony of *D. limbata* could not be established. Deficiencies in the food, such as too little or too much bacteria (yeast), may have adversely influenced the survival of the lice populations. The quality of the skin squares that are frozen must be good. Fresh skin scrapings are necessary to ensure the vitality of the *D. limbata* colony.

CHAPTER 4

DETERMINING THE NUMBER OF IMMATURE INSTARS OF *DAMALINIA LIMBATA* USING MORPHOMETRIC CHARACTERS.

4.1 Introduction.

Morphometrics is the measurement and analysis of form (Daly, 1985). The form can be virtually anything: a lake basin, sand grain, cellular organelle, ape skull or the form of a phenomenon (Daly, 1985). Early biometricians recognized that insects are advantageous subjects for studies of variation because the exoskeleton is easily measured and largely free of physical distortions suffered by the soft bodies of many other animals (Daly, 1985). The use of frequency distributions to determine the number of nymphal stages or instars is a long established technique in entomology and other zoological disciplines (Dyar, 1890; Taylor, 1931; Bhattacharya, 1967; Daly, 1985).

Postembryonic growth of insects, i.e. increase in size, is discontinuous, i.e. major increases are limited to the periodic moults of the cuticle, therefore the sclerotized structures of an individual insect are usually assumed to remain constant in size during any given instar (Daly, 1985). The consistency in size of the sclerotized structure is, however, largely dependant on the degree of sclerotization because darker more sclerotized structures has less elasticity than lighter less sclerotized structures (Daly, 1985). It is therefore important to choose well sclerotized characters to minimize the influence elasticity may have on the accuracy of measurements. The structure most commonly used is the head capsule where the widths or the linear distances between landmarks are measured (Dyar, 1890; Kishi, 1971; Mackay, 1978; Floater, 1996).

The number and identification of each of the instars are important aspects of life history studies and may be of practical significance in pest

management (Daly, 1985). The approach that provides the most understanding is to combine laboratory rearing of individual insects together with the analysis of collections of nymphs taken in the field (Daly, 1985). For expediency or because the immature instars can not be reared in the laboratory, the desired information is often obtained from field collections alone (Hynes & Hynes, 1975; Mackay, 1978; Daly, 1985).

In this study the head capsule widths and lengths were used in order to determine the number of instars of *D. limbata* from feral lice. Laboratory colonization of *D. limbata* was unsuccessful (see Chapter 3) and comparisons between individuals from field populations and individuals from laboratory reared populations were therefore not possible.

4.2 Material and methods.

Lice were collected from Angora goats in the field. Mohair cuts of three heavily infested goats were taken to the laboratory and placed under a bright light in a Berlese funnel. These lice are negatively phototactic and therefore move downward, away from bright light, until they fall into a funnel under the mohair and are collected in a beaker containing 70 % ethanol. The lice are thus preserved and can be measured at any time, without the dilemma of distortion of body features due to desiccation.

The most sclerotized areas on the lice are the head capsules and were, as such used for the measurements. The measurements of the head-capsule widths were taken as the distance between the antenna. This distance was used instead of the wider part of the head anterior of the antennae, because the trabeculae are situated here which are movable and therefore not suitable for measurement. The length of the head capsule was regarded to be the distance between the anterior edge of the head visible from above (the middle of the frontal notch) and the posterior edge of the head (Fig.4.1.). In total 249 lice were measured with a Zeiss dissection microscope containing a calibrated ocular at 40X magnification.

A finite mixtures analysis (FMA) was done on the head-capsule measurements. The head-capsule widths and head capsule lengths were analyzed separately using the FMA-N1© software written by Dr John Randal[1]. FMA resolves a multimodal curve of a frequency distribution into its individual Gaussian components and calculates the proportions for each component in areas of overlap, i.e. it calculates the normal distribution curve for each peak in a frequency distribution (Flury & Randal, 1995).

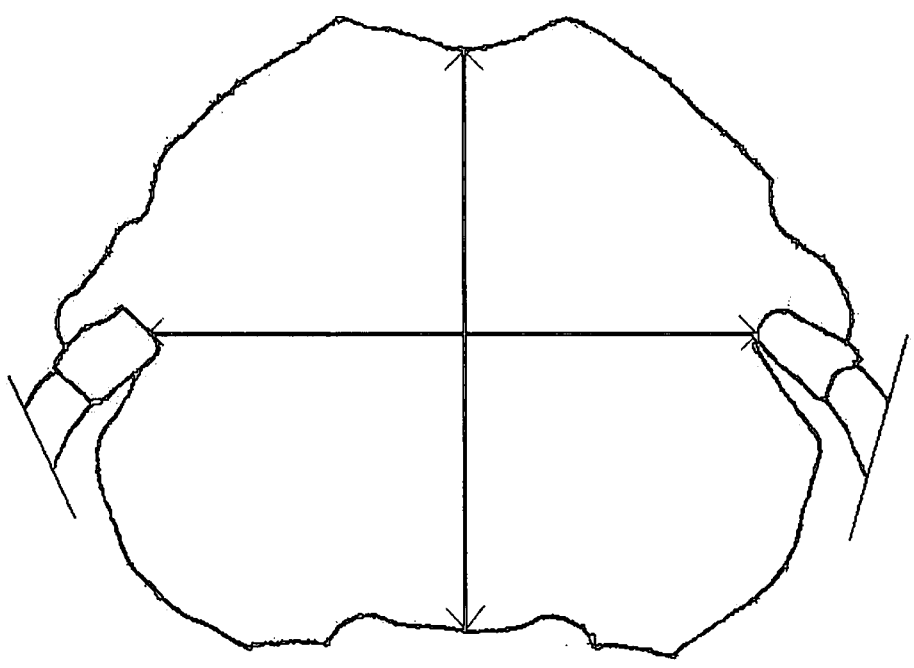

Fig. 4.1. Schematic representation of the head-capsule of *Damalinia limbata* with the lines of measurement indicated by the arrows.

The data obtained from the FMA (i.e. the classification of each measurement into groups 1, 2 or 3) were tested to determine whether there are any missing instars using the Brooks-Dyar rule (Dyar, 1890; Daly, 1985; Floater, 1996). The rule states that nymphal head widths in successive stages describe a regular geometric progression (Dyar, 1890) with the following equation:

[1] Dr. John Randal, Dept. Economic Agriculture, Univ. Stellenbosch, Stellenbosch, South Africa.

$$Y = a(e^{bX}) \qquad (1)$$

where X is the instar number (1, 2, 3, etc.); Y is the head-capsule width (or length); and "a" and "b" are constants. The equation serves both as a growth curve and as a method of checking for an overlooked instar in a frequency distribution (Daly 1985; Floater, 1996). To check for a hidden instar, equation (1) is made linear by taking the natural logs of both sides, giving:

$$\ln Y = c + bX \qquad (2)$$

where c = ln *(a)*. The relationship between ln Y and X should be a straight line with slope b, and therefore a significant deviation from a straight line indicates a missing instar (Dyar, 1890; Daly, 1985; Floater, 1996).

4.3. Results and discussion.

The 249 measurements of the head widths of *D. limbata* were plotted in a frequency distribution plot to see if there were any visible peaks. Three distinct peaks were visible, which would suggest that *D. limbata* has three nymphal instars (Fig. 4.2.). There were, however, a degree of overlap between the instars and it was therefore difficult to determine where one instar ends and the other starts. To resolve this multi-modal graph into its Gaussian components the data were put through a finite mixtures analysis (Flury & Randal, 1995).

The proportion of each group (instar), represented by the peaks in the multi-modal distribution, in the total population, the average head widths, variances and standard errors, which were calculated by FMA, are given for each instar (Table 4.1). There were no differences between the variances of the three groups and the variances were therefore taken to be equal.

Instar 1 had an average head-capsule width of 0.252mm (± 0.003) and the average head-capsule width of instars 2 and 3 were 0.364mm (± 0.003) and 0.467mm (± 0.004) respectively.

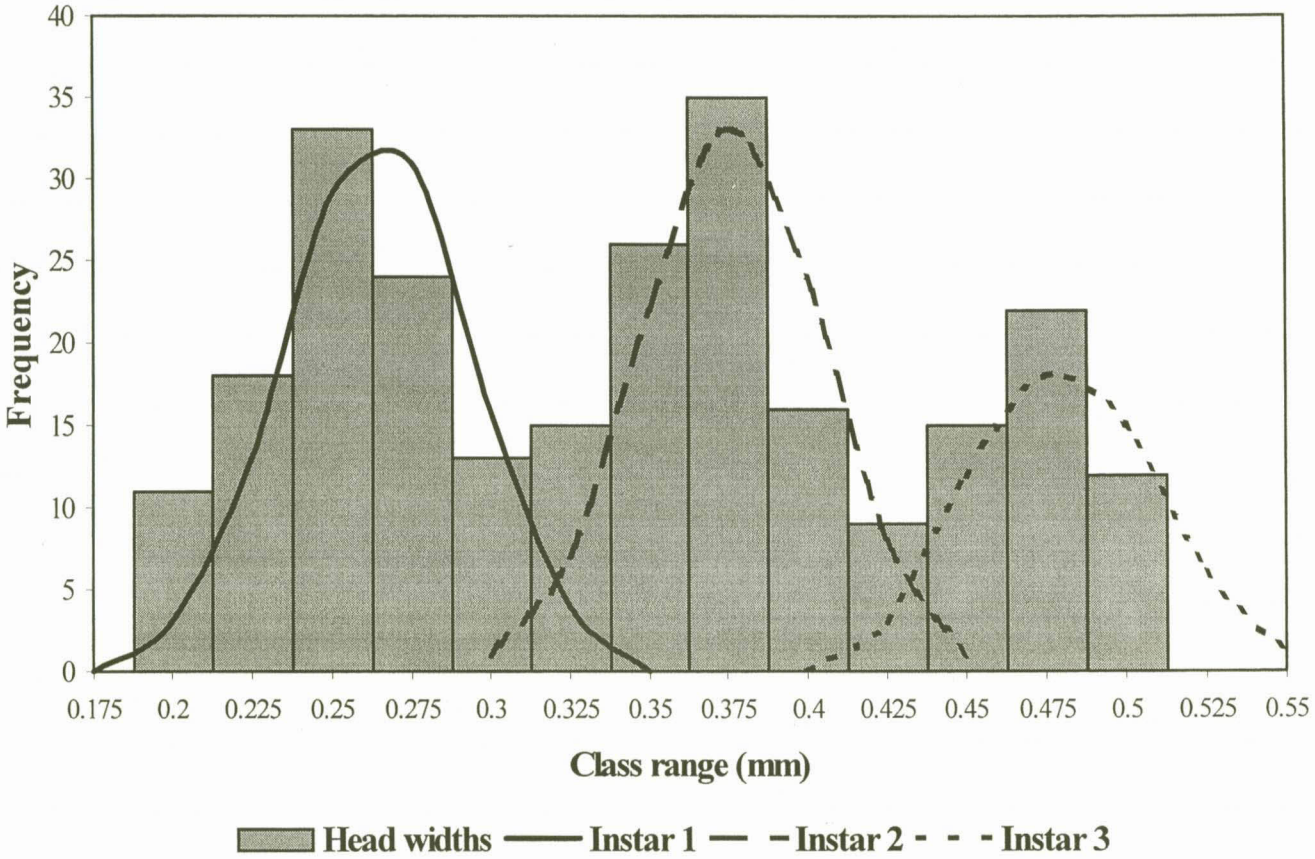

Fig. 4.2. The calculated distribution, of the individual instars in a frequency distribution, of the head widths of *Damalinia limbata*.

Table 4.1. The finite mixtures analysis (FMA) on the measurements of the head-capsule widths of *Damalinia limbata*.

Instar	Proportions (%)	Average head width (mm)	Variance	Standard error
Instar 1	38.93	0.252	0.001	0.003
Instar 2	38.56	0.364	0.001	0.003
Instar 3	22.51	0.467	0.001	0.004

The values of the separate curves for each instar were calculated using the density function of the normal distribution (Fig. 4.2).

$$f(y;\mu_2,\sigma^2) = \int_{-\infty}^{+\infty} \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\bar{x}}{s}\right)^2} \qquad (3)$$

Where x is the specific head width, $\bar{x}$ is the average head width of the instar and S is the square root of the variance.

It is clear from Fig. 4.2 that there is a reasonable degree of overlap between the three instars of *D. limbata*. The calculated range of instar 1 is from 0.175 – 0.35 mm; instar 2 ranges from 0.3 – 0.45 mm and instar 3 ranges from 0.4 – 0.55 mm. Instar 1 and 2, however, has an overlap between 0.3 – 0.35 mm and instar 2 and 3 overlap between 0.4 – 0.45 mm. As such, only individuals with head capsules narrower than 0.3 mm can be classed as first instar nymphs with any confidence. The same applies to second and third instar nymphs, where the reliable ranges are from 0.35 – 0.4 mm and larger than 0.45 mm respectively.

In the areas of overlap, the Fma-N1 program also calculates the likelihood of the specific measurement belonging to either of the two overlapping groups. It was found that, in the area of overlap between the first and second instars, an individual with a head width of 0.3 mm has a 100 % probability of being a first instar nymph and a zero percent probability of being a second instar nymph, while an individual with a head width of 0.325 mm has a 8.53 % and 91.47 % probability of being first and second instar nymphs respectively.

A frequency distribution was also plotted for the head lengths of *D. limbata* and similar results to those of the head widths were obtained

(Fig 4.3.). The modes of the frequency distribution of head lengths, however, are not as symmetrical as those of the head widths.

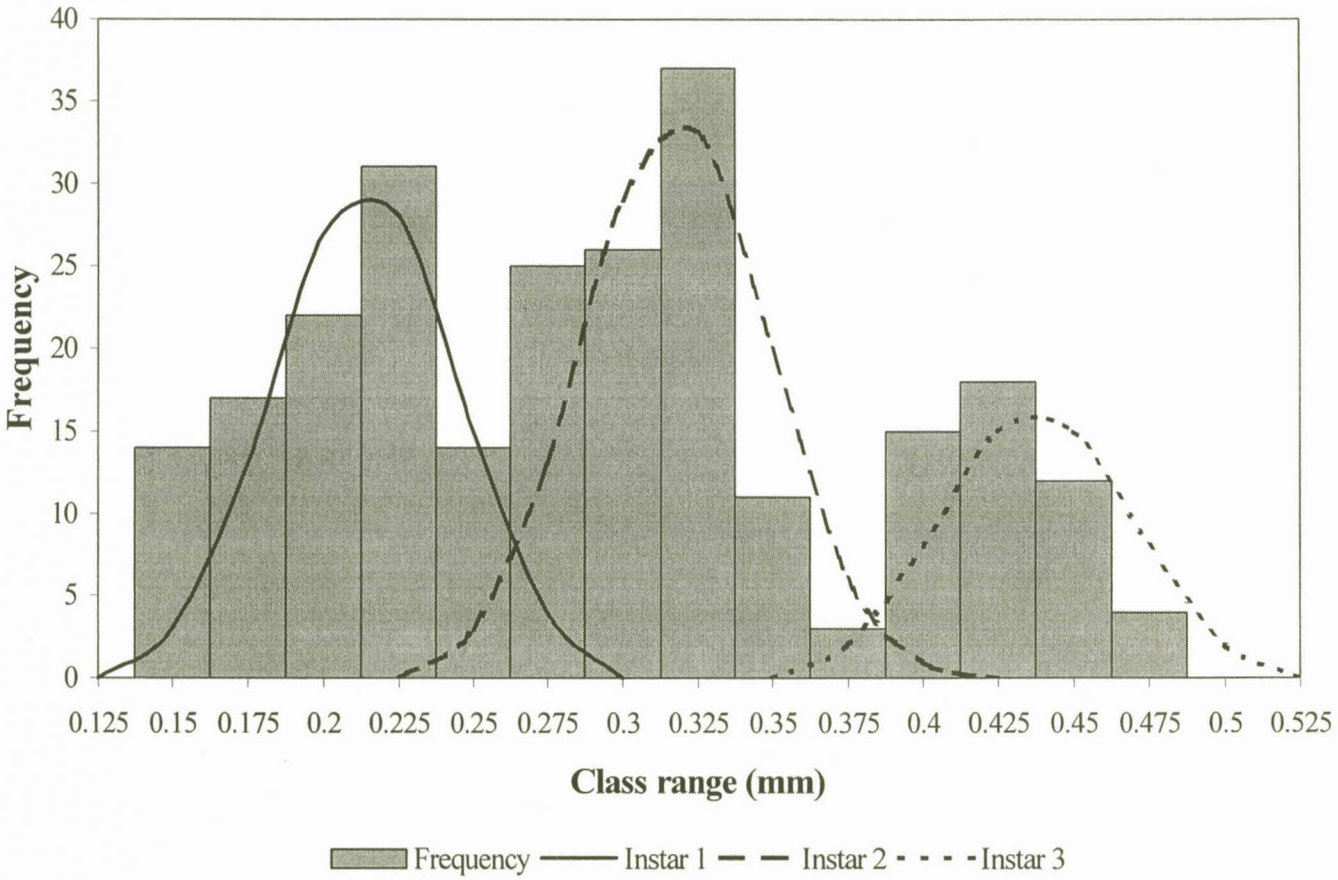

Fig. 4.3. The calculated distribution of the individual instars in a frequency distribution of the head-capsule lengths of *Damalinia limbata*.

Table 4.3. The finite mixtures analysis (FMA), on the measurements of the head-capsule lengths of *Damalinia limbata*.

Instar	Proportions (%)	Average head length (mm)	Variance	Standard error
Instar 1	37.16	0.202	0.001	±0.003
Instar 2	42.19	0.305	0.001	±0.003
Instar 3	20.66	0.425	0.001	±0.004

A FMA was also performed on the head lengths and the proportions, average head lengths, variances and standard errors are given for each instar (Table 4.3). The variances of the three instars were also found not to be different and were therefore considered equal.

The density function (formula 3) was again used to calculate the separate normal curves for each instar (Fig. 4.3). From Figure 4.3. it is evident that there is a reasonable degree of overlap between the three instars of *D. limbata*. The first instar ranges from 0.15 – 0.275mm; instar 2 ranges from 0.25 – 0.4mm and instar 3 ranges from 0.375 – 0.5mm. Instar 1 and 2, however, has an overlap from 0.225 – 0.3mm and instar 2 and 3 overlap from 0.35 – 0.425mm. Therefore, only individuals with head lengths shorter than 0.25mm can be classed as first instar nymphs with any confidence. The same applies to second and third instar nymphs, where the reliable ranges are from 0.3 - 0.35mm and larger than 0.425mm respectively.

The results of the two FMAs were tested with the Tukey test. This was done to see if the groups identified by FMA were indeed significantly different. The Tukey tests ($p=0.05$) done on the head capsule widths showed that the three groups were indeed statistically very different, even with the areas of overlap included (Table 4.3).

The Tukey test ($p=0.05$) done on head capsule lengths gave the same results as with the head capsule lengths (Table 4.3). This would seem to indicate that the FMA calculations were accurate and can thus be used to determine the number of immature instars of an insect.

The proportions of the three instars in both head-capsule widths and lengths in the two data matrices are very similar. This indicates that both head width and head length can be used to determine the developmental stages of *D. limbata* collected from the field. The overlap between the instars, however, causes a percentage of the population to be unidentifiable through uncertainty as to which group it belongs. The

phenomenon of overlap between instars is however, common in insects, and a variety of reasons for its occurrence have been advanced (Daly, 1985). Growth rate may not be constant for the population of nymphs sampled. There may be individual variation in growth rate (Kishi, 1971; Asante & Cairns, 1995), or there may be sex differences in growth rate and morphometrics (Floater, 1996). No growth may occur in one or even more of the instars (Taylor, 1931; Floater, 1996).

Table 4.3. Results of the Tukey test done on the head capsule widths and lengths obtained from the FMA, to determine the instars are significantly different.

	Difference ξ_B-ξ_A	SE	Calculated q	$q_{(0.5;248;3)}$ [φ]	Conclusions
Head width					
Inst. 1vs2	0.112	0.003	38.538	3.314	Instar1 ≠ Instar2
Inst. 2vs3	0.103	0.003	30.249	3.314	Instar2 ≠ Instar3
Inst. 1vs3	0.215	0.003	63.142	3.314	Instar3 ≠ Instar1
Head length					
Inst. 1vs2	0.103	0.003	33.655	3.314	Instar1 ≠ Instar2
Inst. 2vs3	0.120	0.004	32.402	3.314	Instar2 ≠ Instar3
Inst. 1vs3	0.223	0.004	59.109	3.314	Instar3 ≠ Instar1

[φ] Obtained from Table B5 in Zar (1984).

Errors in taking the measurements, such as rounding error due to the researcher making a mistake or the measuring instrument not being sensitive enough may also lead to an overlap between instars (Daly, 1985). The data in this study has a strong bias towards increases in size of 0.025 mm. This was probably a result of rounding error due to the reasons mentioned above.

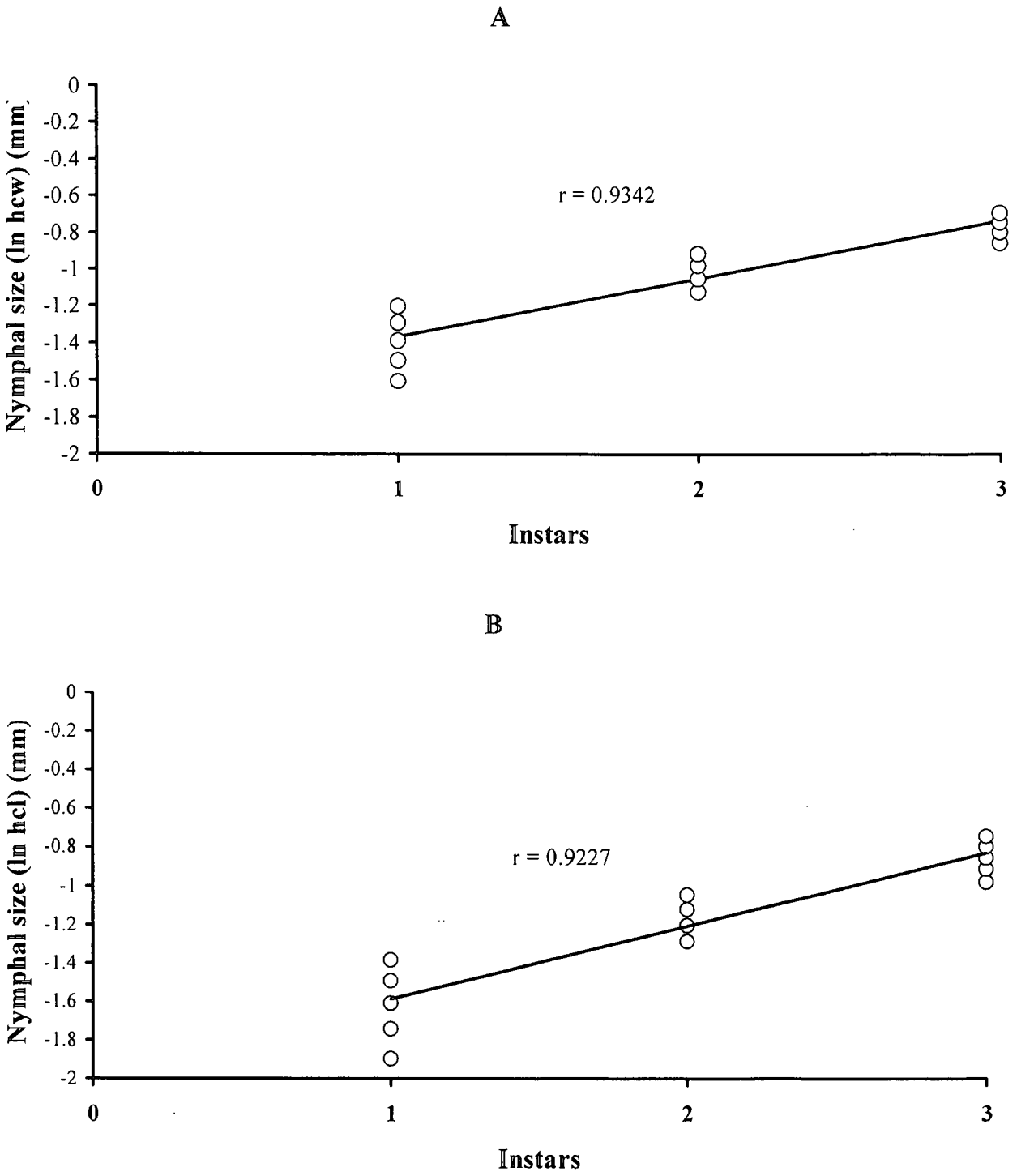

Fig. 4.4. A regression showing the geometric increase of nymphal size *Damalinia limbata* (represented as ln [head-capsule width or length]) with corresponding instar number: (A) head-capsule width (hcw), (B) head-capsule length (hcl).

A regression analysis was done on the data for both head-capsule widths and lengths to test for missing instars. The class interval for each instar was based on the results of the FMA i.e. if FMA classified a measurement as first, second or third instar it was accepted as such. The following categories were created: head-capsule width: instar 1: 0.2 – 0.3 mm; instar 2: 0.325 – 0.4 mm; instar 3: 0.425 – 0.5 mm; head-capsule length: instar 1: 0.15 – 0.25mm; instar 2: 0.275 – 0.35 mm; instar 3; 0.375 – 0.475 mm.

A regression line was plotted for each of these dimensions against the corresponding instars (Fig. 4.4.). The result is a near perfect straight line in both instances. The regression equation for the head-capsule width is: $Y = 0.3153X - 1.6809$; $r = 0.9342$ and consequently the three peaks do seem to correspond to three consecutive instars corroborating the findings of Hopkins & Chamberlain (1969).

The same was found to be true for the head-capsule lengths with the regression equation: $Y = 0.3798X - 1.9689$; $r = 0.9227$. Because the increase in size is geometric from one instar to the next, the size ratio of one instar to the next should be constant (e^b). This ratio is 1.37 for head-capsule width and 1.46 for head-capsule length, the so-called Dyar's law (Dyar, 1890; Taylor, 1931). The range of head-capsule widths and lengths around the mode does not seem to get progressively larger as the nymph moults from one instar to the next (Fig. 4.4.). This can be attributed to rounding error limiting the variation (Daly, 1985).

When the averages calculated by FMA are used, however, the size increase obtained from one instar to the next is not geometric for either head-capsule width or length. The ratios for head-capsule width increase from instar 1 to 2 and from instar 2 to 3 are 1.44 and 1.28 respectively and for the head-capsule lengths the increases are 1.51 and 1.39 respectively. Theoretically the Brooks-Dyar rule should hold for any insect (arthropod) provided marked increases in head size occur during moults and only then (Taylor, 1931; Daly, 1985). It is, however, necessary to rear the insects in

the laboratory and follow the development of each individual if possible (Taylor, 1931; Mackay, 1978; Daly, 1985; Floater, 1996).

The Brooks-Dyar rule remains controversial: it serves as a practical guide for the number of instars of some insects, such as Lepidopterous nymphs (Dyar, 1890), sawfly nymphs (Taylor, 1931), some species of Trichoptera (Mackay, 1978) and the processionary caterpillar, *Ochrogaster lunifer* Herrich-Schäffer (Floater, 1996). For some other insects, however, this law does not hold: e.g. the pine bark weevil, *Pissodes nitidus* (Kishi, 1971) and nymphs of the Tortrichid, *Choristoneura viridis* (Schmidt *et al.*, 1977). The reasons for the varying degrees of successes, obtained with the use of Brooks-Dyar rule specifically and morphometrics in general, have been discussed by various authors. Schmidt *et al.* (1977) and Daly (1985) concluded that the usefulness of the Brooks-Dyar rule may be limited by developmental polymorphism i.e. the number of instars may differ between the sexes or vary among individuals. Asante & Cairns (1995) also found that the morphometric aspects of the wooly apple aphid, *Eriosoma lanigernum* (Hausmann), varied greatly between populations from different areas as well as within populations located in the same area. They also stated that environmental factors, such as variation in temperature and food supply, might also influence the morphometric dimensions of an insect within a population.

4.4 Conclusion.

Damalinia limbata has three instars during its nymphal development. Whether the increase in size from one instar to the next follows a geometrical progression could not be determined with any certainty. Field data should be used in conjunction with laboratory reared colonies for greater accuracy. The sensitivity of measurements should be increased to limit the effects of rounding error and possibly obtain a better separation of the frequency distribution.

CHAPTER 5.

SEASONAL POPULATION FLUCTUATIONS

5.1 Introduction.

Lice are generally not considered economically important ectoparasites of wild life (Matthee *et al.*, 1998). However, large numbers of lice are frequently recorded on young and old animals in poor health and animals suffering from stress related conditions (Kettle & Lukies, 1982b). Lice, however, have been associated with loss of wool production and quality on sheep (Kettle & Lukies, 1982a; Wilkinson, *et al.*, 1982). It seems as if the same phenomenon occurs with *Damalinia limbata* on Angora goats (see Chapter 6.).

A sound knowledge on the seasonal population fluctuations of parasites is a prerequisite to formulate effective control strategies. Very little is known about the ecology and the predilection sites of *D. limbata on* Angora goats. Work on a related species was done by Murray & Gordon (1969), who found that *D. ovis* populations on sheep increased and decreased in abundance annually. It was found that *D. ovis* numbers declined in spring, stayed low during summer and increased during autumn and winter (Murray & Gordon, 1969; Zumpt, 1970). Murray (1960) studied the influence of temperature on populations of *Linognathus pedalis* and found that the temperatures in the micro-habitat of the lice may vary greatly. The intensity of solar radiation during the Australian summer might increase the temperature near the tip of the fleece of sheep up to 70-80 °C (Murray, 1968).

The same factors may also influence *D. limbata* populations on Angora goats. In this study the seasonal changes in the populations of *D. limbata* and the environmental factors that may be responsible for these changes were therefore studied. Specific emphasis was placed on the distribution of lice on the body of Angora goats.

5.2 Material and methods.

5.2.1. Study site.

The study was conducted on the farm Preezfontein (29°50'S, 25°19'E), situated 10 km from the town of Fauresmith, about 130km south-west of Bloemfontein in the south-western Free State. The veld type of this area is defined as 'False Upper Karoo' and falls within the Karoo biome (Acocks, 1988).

5.2.2. Data.

The long-term (20-years) average rainfall for the area is ca; 480 mm, occurring mainly during autumn and spring (Fourie, *et al.*, 1995). The rainfall in this area can be highly erratic and periodically gives rise to mild or even severe droughts. The daily and seasonal air temperature fluctuation can be tremendous and the absolute temperatures may vary between –10 °C and 36 °C.

The daily environmental minimum, maximum and average temperatures and minimum, maximum and average relative humidity (RH) were measured with a data-logger (MCM systems, Cape Town) stationed at the study site over a period of one year, from May 1994 until April 1995.

5.2.3. Material.

Ten adult goats, consisting of wethers and non-gravid ewes, were selected from a larger herd. The goats were placed in an enclosed area (about 2 ha.) with enough food and water to sustain them. Mineral supplements in the form of salt licks were supplied periodically.

The goats were monitored for lice infestations at monthly intervals. The lice of each goat were counted on one side of the body on the following areas: (1)

shoulder, (2) brisket, (3) neck, (4) flank, (5) thigh, (6) groin and (7) abdomen (Fig.5.1).

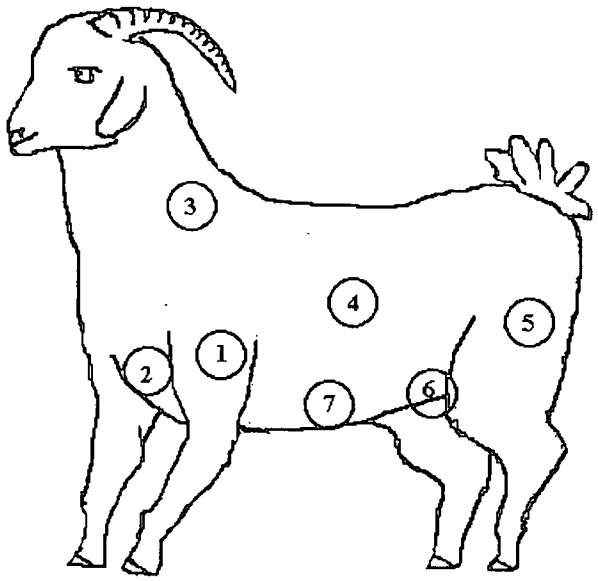

Fig. 5.1. Sites on the body of an Angora goat where *Damalinia limbata* counts were made.

Table 5.1. Values assigned to nymphal estimates

Estimate	Value
0	0
1-10	10
11-50	50
51-100	100
101-150	150
151-200	200
>250	250

Lice were divided into two groups: (1) adults and (2) nymphs. All adult lice in the recording area were counted. Concurrently with counts of adult lice, estimates of nymphal numbers were used (all stages are visible to the naked eye) and assigned values as shown in Table 5.1.

5.2.4. Sampling techniques.

Several sampling techniques were investigated in an attempt to obtain a standardised method for all areas monitored. At first strips of adhesive tape were tested. A piece of tape was cut into 10 cm lengths. One length of tape was placed on each of the hair partings where the lice were monitored. The expected result was that the lice would then adhere to the tape. This method, however, did not work well because the natural oils found in the mohair counteracted the adhesive properties of the tape. Also, the population dynamics of the lice may be influenced unnaturally, by removing the lice from the goat. Therefore a more dependable technique had to be developed to avoid these problems. An aluminium frame was constructed with an observation area 2 cm wide by 5 cm long. This technique ensured a standard observation area of 10 cm^2. The frame was placed on the site of investigation and the lice in the standard area were counted according to the methods sited in 5.2.3. This method ensured that the lice remained in the area that was being inspected, because the lice under investigation tended to move away from strong light during counting.

Counts of adult lice, or nymphal estimates, were totalled for the seven body areas of a particular goat. The average values for the group thus denotes, for a specific date, average totals for the seven body areas investigated.

5.3. Results and discussion

5.3.1. Weather data

The average, minimum and maximum temperature (°C) and average, minimum and maximum relative humidity (%RH), of the study site, were calculated for each month (Table 5.2.). The lowest average temperature was for the month of July 1994 (4 °C), but the lowest minimum temperature was recorded on the 8 June 1994 (-10 °C). The highest average temperature was recorded during February 1995 (22.7 °C). However, the highest maximum temperature was

recorded on 6 January 1995 at 35.9 °C.

The lowest average RH was for the month of September 1994 (32.1%), but the lowest minimum RH was recorded on the 8 July 1994 (0%) (Table 5.2.). The highest average RH was recorded during June 1994 (67%). However, the highest maximum RH was consistently above 90% for all months, except during September 1994, when a maximum was reached on the 18 September at 88.7%.

Table 5.2. The monthly, average, minimum and maximum temperature and the average, minimum and maximum RH for the study site, Preezfontein.

	Avg. monthly temp. (°C)	Lowest min. temp. (°C)	Highest max. temp. (°C)	Avg. monthly RH (%)	Lowest min. RH (%)	Highest max. RH (%)
May '94	8.7	-9	24.2	53.1	15	>98
Jun. '94	4.6	-10	18.7	67	22.2	>98
Jul. '94	4	-9.5	19.2	48.4	0	>98
Aug. '94	7.1	-7.3	24.4	46.1	12.2	>98
Sep. '94	12.5	-4.6	27.5	32.1	10.7	88.7
Oct. '94	15.6	-2.5	30.1	36	10.1	91.6
Nov. '94	17.6	1.7	32.8	39.4	8.22	>98
Dec. '94	20.4	4.4	34.8	36.6	11	94.3
Jan. '95	21.6	8.2	35.9	41	11.3	>98
Feb. '95	22.7	9.2	35.5	43.2	12.2	>98
Mar. '95	17.2	5	31.3	64.7	12.5	>98
Apr. '95	13.7	-2.1	27.8	58.3	12.3	>98

5.3.2. The influence of temperature on population fluctuation.

The seasonal variation of populations of adult *D. limbata* are illustrated in Figure 5.2 with the average environmental temperature, for the period May 1994 to April 1995. Adult *D. limbata* populations decreased from an average of 22 lice/seven areas totalled, to 8 lice/seven areas totalled, during the period from June to July. The goats were shorn in July, before the counts were made, and this would account for some of the drop in lice numbers. The lice population increased slightly during the following two months and peaked in October. The goats were shorn again in February resulting in a slight decrease in population numbers from an average of 33 lice/ seven areas totalled, before shearing in

January to an average 25 lice/ seven areas totalled, after shearing. Low average temperatures persisted during the first months of the study. From an average of 8.7°C in May the temperature dropped to an average low of 4 °C in July and increased to 7.1 °C in August after which it increased gradually until February when it reached a peak at an average of 22.7 °C (Fig. 5.2).

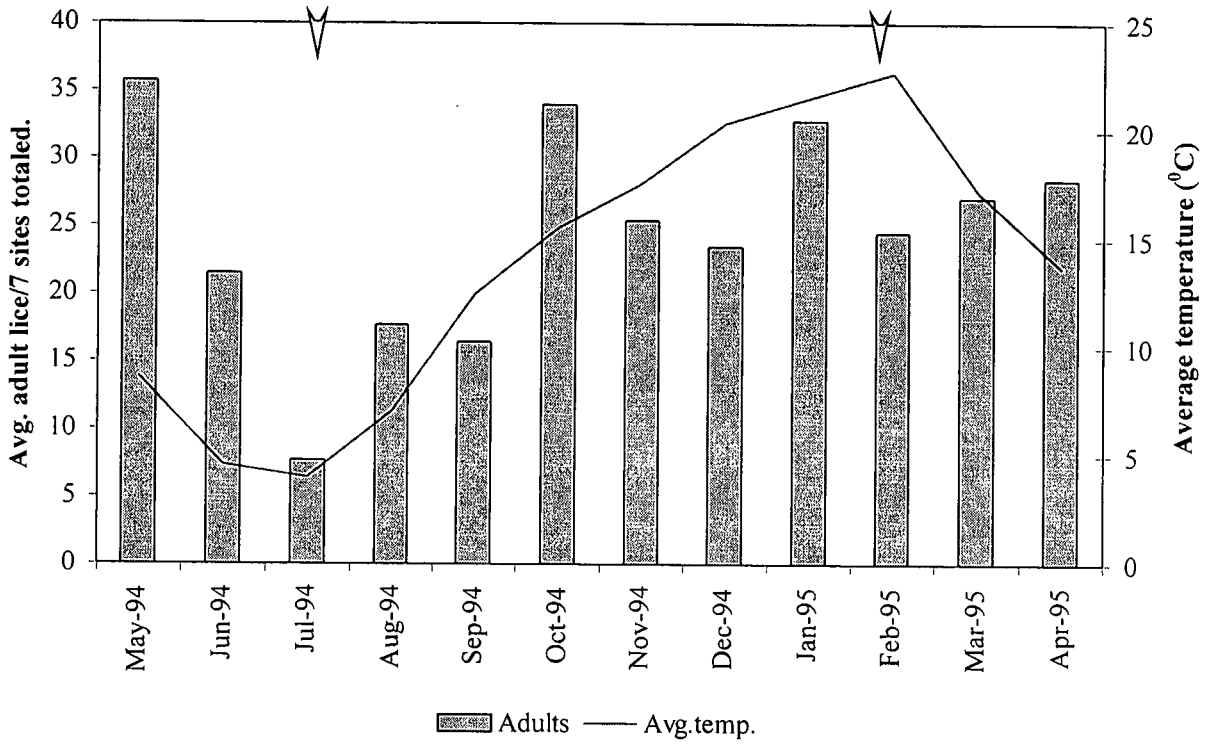

Fig. 5.2. The average number of *Damalinia limbata* adults per seven site totaled and the average temperature over a one year period. The arrows indicate shearing.

Population numbers of *D. limbata* nymphs decreased from May to July (when the goats were shorn) and then increased slowly over the next six months and peaked in January at an average of 321 lice per goat, just before the goats were shorn again (Fig. 5.3). Peak nymphal numbers were reached three months after that of the adult populations (compare Figs. 5.2 and 5.3).

A decrease in the population size of nymphs occurred again after shearing in February (the lice counts were made after shearing) and continued to decline until March. Decline of nymphal population numbers during February was less

pronounced than in July.

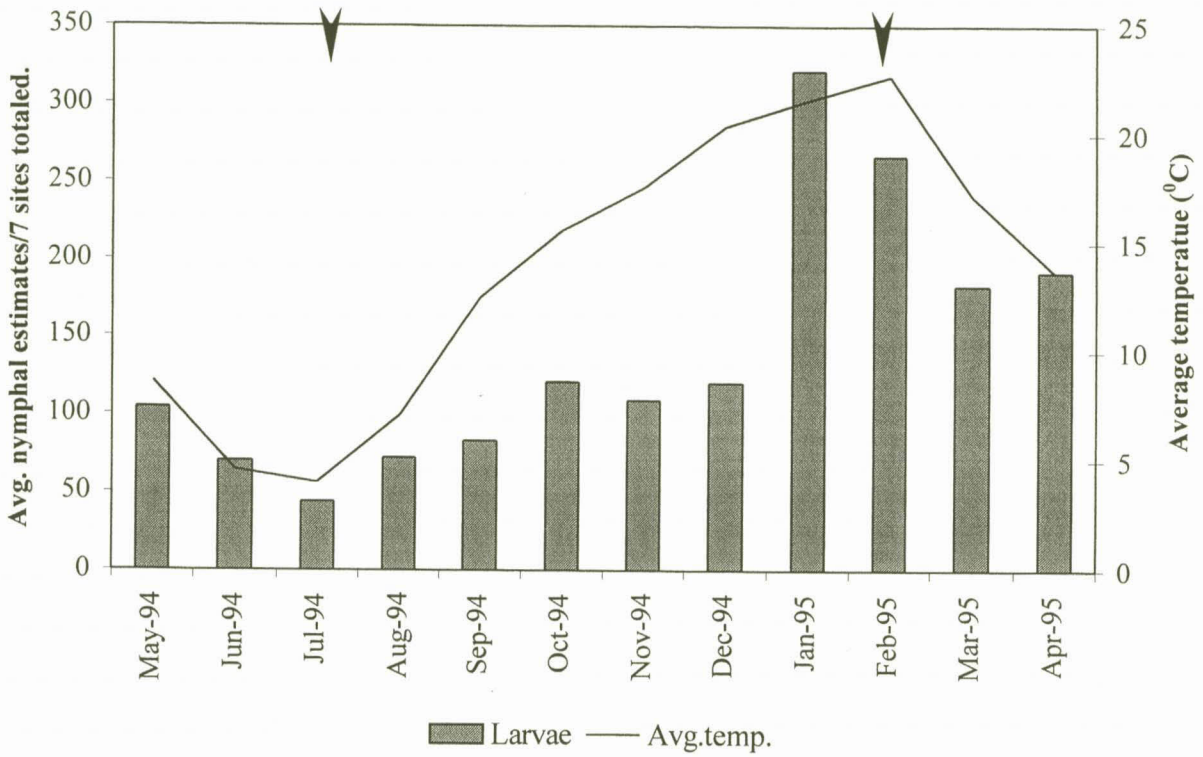

Fig. 5.3. The average estimates of *Damalinia limbata* nymphs per seven sites totaled and the average temperature over a one year period. The arrows indicate shearing.

The decrease in *D. limbata* populations due to shearing correlates well with results obtained by Kettle & Lukies (1982b), Wilkinson, *et al.* (1982) and Niven & Pritchard (1985) who also reported that shearing greatly reduced *D. ovis* populations on sheep. Murray & Gordon (1969) reported that *D. ovis* populations, on sheep, could be reduced by 30-50 % as a result of shearing. Although the adult population of *D. limbata* decreased by between 60-70 % after shearing in July the nymphal population only decreased by between 30-40%. The combined adult and nymphal population reduction would, therefore, probably be closer to that reported by Murray & Gordon (1969). It was, however, not possible to combine the data for adult and nymphal lice due to the difference in counting methods. However, the decrease in population numbers of *D. limbata* cannot be attributed to shearing alone. Both adult and nymphal populations decreased during winter, before shearing (Figs. 5.2 & 5.3), as the environmental temperature became progressively lower. It would seem, therefore, that

environmental factors, such as temperature, might be as important as shearing in lowering population numbers.

In Australia, louse (*D. ovis*) populations on sheep decrease in late summer after shearing but increased during the winter (Murray 1963, Murray & Gordon 1969). However, this did not hold true for *D. limbata*, which increased during spring, after shearing and peaked during early spring and mid-summer for adult(Fig. 5.2) and nymphal (Fig. 5.3) populations respectively and then remained reasonably constant. Murray & Gordon (1969) attributed the decline of lice in summer to shearing, solar radiation and thunderstorms. Heavy rainfall was very rare during the current study and would therefore not have had a great influence on the lice populations. Wilkinson *et al.* (1982) noted that Murray & Gordon (1969) based their conclusion about solar radiation on the observations of Murray (1968) who used sheep with fleece 2.5 – 5 cm in length. The goats in the current study were carrying mohair 6 – 7 cm in length when first exposed to the high levels of solar radiation in late spring but the hair was as long as 13 cm just before shearing in late summer. The long mohair would give greater protection to the lice against high levels of solar radiation ensuring greater stability in the lice population size. This view is supported by Wilkinson *et al.* (1982) and Niven & Pritchard (1985), who found that *D. ovis* populations did not decline during summer on sheep with long fleece.

The sharper decline in lice populations after shearing in the winter compared to summer can be attributed to lice moving up and down in the mohair more actively in winter than in summer due to lower levels of solar radiation and lower ambient temperatures. The result was that more lice were removed with the mohair when the goats were shorn. This is in agreement with Murray (1968) who found that *D. ovis* on sheep, were negatively phototactic and tend to move away from strong light. In summer, when the radiation levels are high, the lice tend to stay closer to the skin and tend to move to more shaded areas (Murray, 1960). *D. limbata* is also negatively phototactic and will therefore move around more in winter during the day when solar radiation is lower, but the temperatures more

suitable to the lice. More lice will therefore be removed from the goats, by shearing, at times when they are not close to the skin but more widely dispersed through the mohair. This situation is more prevalent during the winter.

During July the extreme temperatures varied between –9.5 °C and 19.2 °C. These severely cold conditions experienced during the winter months in central South Africa, could cause further mortality after shearing, because the lice are more exposed to the greatly fluctuating temperature when the goats have short hair. The temperature gradient that is prevalent on goats with short mohair is much smaller compared to goats with long mohair (see Chapter 3). This supports the view of Murray & Gordon (1969) who stated that the major effect of shearing was the alteration of the habitat so that the microclimate becomes more variable.

The delay in the nymphal population build-up compared to the adult population can be attributed to adult populations being too low after the mid-winter shearing to ensure a fast recovery in nymphal populations. Murray & Gordon (1969) stated that, due to the low reproductive potential of *D. ovis* on sheep, the ultimate size of the population is greatly influenced by the size of the population after shearing and the length of time that favourable conditions prevail.

The average number of adult lice/10 cm^2 area decreased from 3.1 in June to 1.1 during July after shearing and the nymphal populations from 10 in June to 6.3 in July. This is very low and the subsequent recovery of the lice populations will be slow. After shearing in summer, however, the average number of adult lice, per 10 cm^2 area, decreased from 4.7 in January to 3.5 in February and the nymphal populations from 45.9 to 38.1. The subsequent recovery of the lice populations will, therefore, be more rapid after the summer shearing than after the winter shearing.

5.3.3. Spatial distribution of *Damalinia limbata* on the body of Angora goats.

The lice data were pooled for each season and the average values calculated:

winter was from June-August, spring from September-November, summer from December-February and autumn from March-May. When the data for the four seasons were compared to the body areas it became clear that during winter the abdomen had the highest average number of adult lice at 31.6% (4.9 lice/10cm^2) of the total population (Table 5.3). The flank had the highest average nymphal estimates at 29% (18 nymphs/10cm^2) of the total population (Table 5.3).

The data for spring showed a change in the spatial distribution of *D. limbata* on the body of the goats. Adult lice were more abundant on all areas of the body, but were most abundant in the groin area at 24.4% (7 lice/10cm^2) of the total population (Table 5.3). Nymphal estimates for spring were also generally higher than during winter except for the abdomen, which had declined from an average of 8.7 to 5.9 lice/10cm^2 (from 14% to 5.7% of the total population). The greatest abundance of nymphs was found on the shoulder 22.7% (23.7 nymphs/10cm^2) of the total population (Table 5.3). The neck and flank regions also had high nymphal numbers, with 20.9% and 20.6% (21.8 and 21.5 nymphs/10cm^2 respectively) of the total population respectively (Table 5.3).

During summer the adult lice populations remained at almost the same levels as in spring. The greatest abundance of adult lice was concentrated on the brisket at 25.4% (6.8 lice/10cm^2) of the total population (Table 5.3). The highest nymphal numbers for summer were recorded on the shoulder at 24.8% (58.5 nymphs/10cm^2) of the total population (Table 5.3). Percentage wise this was not much higher than the other seasons which ranged from a low of 22.1% in autumn to the 24.8% (Table 5.3) in summer. In actual lice counts, however, the average number of lice on the shoulder was more than double that of spring and the nymph numbers on most of the body more than doubled (Table 5.3).

The goats were shorn during February (at the end of summer) resulting in a drop in lice population numbers, due to the removal of some of the lice with the mohair, which means that the lice populations might have been higher in the absence of shearing.

Table 5.3. The average number of adult and nymphal *Damalinia limbata* for: Winter, Spring, Summer and Autumn, on the different regions on the body of Angora goats, with the percentage of the population for each.

	Winter Avg. Num.	%	Spring Avg. Num.	%	Summer Avg. Num.	%	Autumn Avg. Num.	%
Adults								
Shoulder	1.5	9.8	3.5	12.3	3.8	14.1	3.8	12.6
Brisket	0.6	3.8	3.9	13.4	6.8	25.4	3.4	11.1
Neck	2.6	16.4	4.8	16.8	4.5	16.7	7.5	24.6
Flank	1.6	10.4	3.0	10.3	3.0	11.3	1.9	6.1
Thigh	0.9	5.5	2.3	8.0	1.5	5.6	1.2	4.1
Groin	3.5	22.4	7.0	24.4	4.8	17.7	7.5	24.6
Abdomen	4.9	31.6	4.2	14.7	2.5	9.3	5.2	17.0
Total	15.6		28.8		26.9		30.4	
Nymphs								
Shoulder	14.0	22.6	23.7	22.7	58.5	24.8	35.2	22.1
Brisket	2.3	3.8	12.2	11.7	36.7	15.6	5.9	3.7
Neck	8.0	12.9	21.8	20.9	51.1	21.7	38.7	24.3
Flank	18.0	29.0	21.5	20.6	35.1	14.9	23.1	14.5
Thigh	6.7	10.8	12.6	12.0	23.3	9.9	28.4	17.8
Groin	4.3	7.0	6.6	6.4	12.2	5.2	10.8	6.8
Abdomen	8.7	14.0	5.9	5.7	19.0	8.0	17.3	10.9
Total	62.0		104.3		235.9		159.4	

In autumn the adult lice populations had increased on the neck and groin and the highest lice loads were on these areas at 24.6% (7.5 lice/10cm^2 for both) of the total population (Table 5.3). The other body areas of the goats, except for the abdomen, showed a decrease in average adult lice numbers. The nymphal estimates of all the body areas also declined after shearing in February and the highest estimates were on the neck and shoulder at 24.3 and 22.1% (38.7 and 35.2 nymphs/10cm^2) respectively of the total population (Table 5.3). During autumn the nymphal populations had declined rapidly in actual numbers except for the thigh, which had increased and had increased from an average estimate of 23.3 nymphs/10cm^2 to 28.4 nymphs/10 cm^2. Percentage wise the nymphal population was more evenly dispersed over the body of the goats (Table 5.3)

Adult lice were found to be proportionally more abundant on the abdomen, groin and neck of Angora goats during winter and most abundant on the brisket, neck and groin during summer. The nymphal populations were highest on the flank and shoulder during winter and during summer the highest nymphal estimates were found on the shoulder and neck. Nymphal populations were also reasonably high on the brisket and flank during the summer.

It would, therefore, seem that the adult populations of *D. limbata* are more numerous on the ventral areas of the Angora goat's body throughout the year. Adult lice seemed to dominate in the groin and abdomen areas during winter. This could be due to the fact that these areas had the highest average temperature on the body of the goat (see chapter 3). Murray (1968) reported that *D. ovis*, on sheep, are attracted to warmth during oviposition, and might also be important when they are hungry. This might also be the case with *D. limbata*. Very low temperatures persisted during July, when the goats were shorn, which would adversely affect *D. limbata*. These extremely low temperatures could cause the lice to move to warmer and more sheltered areas of the body, *viz.* the groin and abdominal areas.

The temperatures during summer regularly exceeded 30 °C and solar radiation could be extreme. In mid-summer the temperature at the tip of the mohair reached as high as 48 °C. It has been found that *D. ovis* on sheep died within an hour, when exposed to these temperatures (Murray, 1968). Because of these lethal temperatures in the mohair the *D. limbata* tend to move away from temperatures which are too high. The brisket does not have dense hair cover and is mostly in the shade. This would, therefore be beneficial to the lice during summer, because it is cool and protected from lethal solar radiation.

The nymphal populations of *D limbata* were most prevalent on the lateral areas of the Angora goat's body throughout the year. The population on the ventral regions did, however, increase as the environmental temperatures increased.

5.4. Conclusions.

A large portion of the lice population was removed from goats during shearing in July and February. The subsequent exposure of the lice to extreme environmental conditions, such as solar radiation and highly variable temperatures, further reduced both adult and nymphal populations of *D. limbata* on all parts of the body of Angora goats. Adult populations seem to be more abundant on the ventral areas of the body and the nymphal populations seem to prefer the dorsal areas of the body.

These factors can be exploited in the regular farming practise and can, in so doing, greatly enhance control of *D. limbata*. Through the application of insecticides after shearing, when lice populations are most vulnerable, more effective control or even eradication can be attained. If chemical control is applied after shearing in winter, the population of *D. limbata* can be reduced to very low levels. If the control were good enough in winter, the lice population would not be able to recover fast enough to be damaging in summer. This is something which can be tested in future studies of these lice.

CHAPTER 6

CHEMICAL CONTROL OF *DAMALINIA LIMBATA* AND THE EFFECT ON BODY MASS AND MOHAIR PRODUCTION OF ANGORA GOATS.

6.1. Introduction

Biting lice of the genus *Damalinia* are a problem of considerable magnitude to small stock farmers throughout the world (Fourie *et al.*, 1995). The negative effects of louse infestations have been quantified for the biting louse *Damalinia ovis* on sheep and include fleece derangement, reduced wool production and a reduction in the processing value of the wool (Wilkinson, *et al.*, 1982). The biting louse *D. limbata* has become a problem in the angora mohair producing areas of South Africa (Fourie *et al.*, 1995). On sheep, biting lice cause discomfort to the hosts as well as negatively affecting the production and quality of the wool (Kettle & Lukies, 1982b; James *et al.*, 1989). *D. ovis* on sheep can apparently affect wool colour, causing lousy wool to be more yellow and in six out of seven fleeces, the colour was less bright than their lice free counterparts (Kettle & Lukies, 1982a). However, very little or nothing is known about the effects of lice infestations on Angora goats.

The control of these lice has become essential for economic and quality control purposes. Various application methods for chemical control agents have been tested and used and plunge dipping has long been the stock method of applying chemical control to sheep and cattle (Anson & Bell, 1971; Keys *et al.*, 1993). However, this method uses large quantities of water and control agent, with a resultant monetary inefficiency due to wastage (Anson & Bell, 1971). Plunge dipping can also have negative effects on the hosts through ingestion and inhalation of the dip mixture (Anson & Bell, 1971). Shower dips and jetting races have been used as well to deliver insecticides (Anson & Bel, 1971; Johnson *et al.*, 1996).

These methods are low volume/high concentration delivery systems that are more cost effective than plunge dipping but they are, however, not always as effective when the host has a long and dense coat (Johnson *et al.*, 1996). Some slow release systems for long term protection against ecto-parasites have also been tested and used (James *et al.*, 1989). Delivery systems, such as insecticide impregnated ear tags and collars provide protection over prolonged periods, avoiding the need for repeated treatments (James *et al*, 1989).

The first pour-on application of an insecticide was probably carried out in 1957 (Johnson *et al.*, 1992). Poultry infested with poultry body lice (*Menacanthus stramineus)* and sheep infested with *D. ovis* were treated successfully by applying a systemic insecticide, Aldrin, to a small area of skin (Johnson *et al.* 1992). According to Levot & Hughes (1990) synthetic pyrethroid (SP) insecticides were introduced for control of lice on sheep in 1981. The chemical is deposited at high concentration in a strip along the back, directly after shearing and disperses around the body of the animal in the wool grease (Levot & Hughes, 1990). A pour-on is a liquid formulation (solution, emulsion, or suspension) intended to be applied to a small area of an animal's skin to promote drug absorption into the blood stream (Kettle *et al.*, 1983). Synthetic pyrethroid insecticides lack systemic effect, however, whereas formulations may be applied in the manner of pour-ons, they are best referred to as backline treatment (Kettle *et al.*, 1983). These insecticides rely largely on peripheral spread of the active ingredient to bring it into contact with the target insect. The eradication of lice from sheep depends on the correct application of an effective lousicide to all sheep in a flock (Morcombe & Young, 1993).

During this study three questions were asked: (1) Is there a relationship between body mass gain and lice burdens? (2) Do lice influence the mass and quality of mohair produced? (3) Is there a significant difference in efficacy of control between a dorsal and lateral application of a lousicide?

6.2. Material and methods.

6.2.1. The effect of *Damalinia limbata* infestation on Angora goat body mass.

To determine the effect of *D. limbata* on body mass, 20 young ewes were selected from a flock of about 200 and weighed to the nearest 0.1 kg. The goats were divided into two groups of 10 using randomisation through minimisation with body mass as the primary criterion. On 19/5/1995 one group was cleared of lice through treatment with undiluted WipeOut[1] (Deltamethrin, 0.5 % m/v), a synthetic pyrethroid (SP) pour-on formulation at 20ml per goat. The other group was not treated and served as a control group. The control group had moderate to heavy lice loads.

The two groups were kept in separate pastures to minimise the possibility of cross contamination between them. Both groups were kept on irrigated pastures with similar vegetation and supplied with salt licks for mineral supplementation. The goats were inspected every two weeks to ensure that the treated group remained uninfested. Three months after treatment the two groups were shorn and weighed. A one way analysis of variance was done on the initial body mass of the treated vs. untreated goats to test for differences. A one way analysis of variance was done on the body mass gain of the treated vs. untreated groups to determine what effect the presence of *D. limbata* had on body mass. Two goats died before the end of the trial, one from each group, therefore the results are based on observations made on the remaining 18 goats.

6.2.2. The effect of *Damalinia limbata* populations on mohair mass and quality.

The same groups of goats used for the body mass trial were used for this trial. All goats were shorn before treatment. The mohair of each goat was kept in a

[1]WipeOut®, Hoechst Ag-Vet, South Africa.

separate bag and transported to the laboratory where it was weighed. After six months the goats were shorn again and the mohair weighed, as mentioned above, and subjectively classed for each group. Mr. A. Du Plessis[2] performed the mohair classing. The mohair cuts were classed according to length: (A) being the longest mohair length (≥150mm); (B) mohair between 125 and 150mm in length; (C) mohair between 100 and 125mm in length; (D) mohair between 75 and 100mm in length and (E) mohair between 50 and 75mm in length. If the mohair had fineness, a hair fibre diameter of less than 36 micron in adult goats, it was denoted with (F). If the mohair had good to super style and character it was denoted with (S). The age of the goat was indicated with (K) for kids, (YG) for young goats and (H) for adult goats. An example would be a cut classed as BFH where the mohair is of a length between 125mm and 150mm long, has fineness and has the characteristics of an adult goat (see Table 6.5).

A one-way analysis of variance was done on the initial mohair cuts to determine whether there was a significant difference in masses of the mohair cuts of the groups. The masses of the initial cuts and the masses of the final cuts of each group were subtracted from each other and a one-way analysis of variance was done on the resultant mass gains or losses thus obtained.

6.2.3. The comparison of two treatment application methods

Two insecticide application techniques were evaluated to establish which is the most effective method. The first was the normal backline treatment where 20 ml of WipeOut was applied to the back of each goat, from neck to tail, with a syringe and the second was applied with the Tickspray[3]unit, a high pressure spray system used to apply WipeOut laterally on the goats. The Tickspray system consists of six, high pressure, nozzles arranged in two groups of three on each side of a stock press. It was calibrated to deliver a dosage of 20 ml of the

[2]Mr. A. Du Plessis, farmer, Langberg, Jagersfontein, South Africa.
[3] Tickspray®, J. L. Viviers & L. Fourie, South Africa, 1995.

insecticide per animal that passes through. As the animals moved through the apparatus it was triggered by breaking a photoelectric beam positioned across the stock press.

In this experiment 24 heavily infested goats were taken out of a large herd. The goats were divided into two groups of ten each and one group of four using the lice burdens as a guide so that all groups had more or less the same level of infestation. The groups were; control group (4 goats); Backline treatment (10 goats) and Tickspray (10 goats). The three groups were kept in separate enclosures, with irrigated pastures and salt licks, to prevent cross contamination between goats. Lice counts were done before treatment on all of the groups, followed by two weekly counts after initial treatment for a period of three months. The lice counts were made at seven sites on the body of the goat *viz.* the shoulder, neck, flank, thigh, groin, stomach and brisket (see chapter 5). A one-way analysis of variance was done on the initial lice counts of all three groups, for adult and nymphal populations, to check whether the lice burdens on the groups are statistically the same. A factorial analysis of variance was done, on all the counts of the different groups for the two months after treatment, to determine whether there were significant differences between the groups and over time.

6.2.4. Statistical methods.

In this study the *F*-test for the analysis of variance approach was used in stead of the *t*-test approach. The *t*-test is essentially the same as the *F*-test used in the analysis of variance, with 1 and *n* degrees of freedom. In other words, when two treatments are being compared the *F* and *t*-tests will give the same result. In fact, the *t*-distribution with *n* degrees of freedom can be derived from the *F*-distribution with 1 and *n* degrees of freedom, because:

$$(t\ df = n)^2 = F\ 1\ ;\ df = 1\ ,\ n.$$

This is illustrated well in the *t*-table and *F*-table in Snedecor & Cochran (1980) on pages 469,480 and 481. Therefore, theoretically for two treatments (for example the two months; Backline vs. Tickspray; Ave. effects of treatment (see Table 6.6).) the analysis of variance approach using *F*-tests will give exactly the same significance levels as the individual *t*-tests. The analysis of variance approach is preferred by statisticians as the error variance (MS Error) needs to be estimated only once, while using individual t-tests it would have to be estimated three times (K. L. Pringle pers. com.). This makes the analysis of variance approach much more efficient. In addition, even when only two treatments are being compared (as in Table 6.3.) the analysis of variance approach is preferred. In this case $F_{1;\ 16} = 4.494$; $P = 0.379$. A t-test gives $t_{16} = 2.120$; $P = 0.379$. Which is the same value for P. The t-test can not be used in cases where there are more than two treatments. Therefore, using only the analysis of variance lends uniformity to the analyses used.

Finally, there are many recent examples of the analysis of variance being recommended by statisticians in instances where only two treatments are compared. For instance in two examples of experimental design Perry in Dent & Walton (1997) compares two treatments in an analysis using the *F*-test for the analysis of variance.

6.3. Results and discussion.

6.3.1. The effects of *Damalinia limbata* infestation on Angora goat mass.

The body mass of the goats in the two groups, as well as mass gained over time, are represented in Table 6.1. The difference in average mass gain between the two groups was only 0.57 kg. The results of a one-way analysis of variance ($P > 0.05$), to determine if the initial body mass of the control and the treated group differed significantly, are represented in Table 6.2. There were no significant

differences ($F_{1, 16}$=1.664; P=0.216) between the initial body masses of the control and the treated groups (Table 6.2.).

Table 6.1. The body mass and mass increase of treated and untreated Angora goats over a six month period.

Goats	Control (kg) (13/2/1995)	Control (kg) (24/8/95)	Control Mass gain (kg)	Treated (kg) (13/2/1995)	Treated (kg) (24/8/95)	Treated Mass gain (kg)
1	15.4	19.2	3.8	18.8	19.4	0.6
2	13.2	16.2	3.0	14.0	19.2	5.2
3	14.0	17.6	3.6	14.4	17.2	2.8
4	11.4	12.3	0.9	15.4	18.0	2.6
5	14.8	15.8	1.0	27.5	28.2	0.7
6	17.0	18.4	1.4	15.2	18.4	3.2
7	16.6	18.2	1.6	19.5	21.6	2.1
8	15.6	17.6	2.0	13.6	18.2	4.6
9	16.4	18.6	2.2	14.6	17.4	2.8
Avg.	14.9	17.1	2.2	17.0	19.7	2.7
SD.	±1.8	±2.1	±1.1	±4.5	±3.4	±1.5

Table 6.2. Analysis of variance of the initial body mass of the control and treated Angora goats groups.

Source of Variation	SS	df	MS	F	P	F crit
Treatment	19.22	1	19.220	1.664	0.216	4.494
Error	184.86	16	11.554			
Total (Corr.)	204.08	17				

A one-way analyses of variance on the body mass gained over time (after shearing) between treated and control groups of goats were done (Table 6.3.). No significant difference ($F_{1;16}$=0.819; P=0.379) in body mass gain between treated and untreated goats were found. *D. limbata* does not seem to adversely affect the body weight of Angora goats.

Table 6.3. Analysis of variance of the influence of *Damalinia limbata* on the body mass of Angora goats.

Source of variation	**Df**	**SS**	**MS**	**F**	**P**	**F crit.**
Between groups	1	1.455	1.455	0.819	0.379	4.494
Error	16	28.220	1.764			
Total (Corr.)	17	29.665				

The goats in this study were stressed due to severe drought during the study period and developed moderate to heavy lice loads, but despite this there were no significant difference in body mass between treated and untreated goats (Table 6.3). These results support those obtained by Kettle & Lukies (1982b) who found that the sheep body louse *D. ovis* had no significant influence on the body mass of sheep (P>0.1). Wilkinson *et al.* (1982) also concluded that it is not valid to attribute any losses in income to changes in body mass of lice infested sheep to the lice infestation it self. Clayton & Tompkins (1995) had similar results on rock doves (*Columba livia*), where lice had no significant influence on body mass and reproductive success. This is probably due to the fact the lice feed on the sebum and scruff in the wool of the sheep and on the feathers of the doves and not the live tissue. *D. limbata*, like *D. ovis*, also feeds on sebum and skin scruff found in the mohair of Angora goats and would, therefore not have a direct influence on the body mass of the goats.

Wounds caused by goats scratching themselves as a result of lice irritation, however, are susceptible to secondary infections that may cause abscesses. These infections may influence body mass and condition adversely. Kettle & Lukies (1982b) noted that the claim that *D. ovis* cause ill-thrift on sheep is probably based on observations of heavy louse burdens on sheep concurrently suffering from some other condition such as parasitic gastro-enteritis. The biting lice may therefore indicate, rather than cause, ill-thrift (Kettle & Lukies, 1982b). This also seemed to be the case with *D. limbata* on Angora goats, where the heaviest lice infestations were usually found on goats with some gastric affliction.

6.3.2. Mohair differences.

The mohair mass of the goats in the two groups, as well as mass gained over time, are represented in Table 6.4. The treated group produced an average of 0.271 kg more mohair per goat (Table 6.4) than the untreated control group over a period of six months. The treated group had an average mohair mass increase of 17.7 % where as the control group had a 5.7 % increase in average mohair mass. At the beginning of the trial there was only a 0.003 % difference in average mohair mass and after a six month period the treated group produced an average of 13 % more mohair than the control group.

The two mohair cuts made on each of the control and treated groups (13-2-95 and again on 24-8-95) were tested for significance in mass gain between the two groups. The results of the analysis, which was done on the mohair mass gain of the two groups, are represented in Table 6.5. The analysis showed a significant increase ($F_{1;16}$=105.329; P<0.0001) in mohair production between the treated and the control group.

Table 6.4. Mohair-mass and mass increase of treated and untreated Angora goats over a six month period.

Goats	Control (kg) (13/2/1995)	Control (kg) (24/8/95)	Control Mass gain (kg)	Treated (kg) (13/2/1995)	Treated (kg) (24/8/95)	Treated: Mass gain (kg)
1	1.780	1.887	0.107	2.007	2.327	0.320
2	1.977	2.002	0.025	1.717	2.001	0.284
3	1.403	1.565	0.162	1.258	1.738	0.480
4	1.499	1.681	0.182	1.861	2.273	0.412
5	1.936	2.033	0.097	1.796	2.169	0.373
6	1.679	1.789	0.110	2.029	2.415	0.386
7	1.768	1.875	0.107	1.742	2.172	0.430
8	1.897	1.956	0.059	1.560	1.958	0.398
9	1.748	1.846	0.098	1.759	2.063	0.304
Avg.	1.743	1.848	0.105	1.748	2.124	0.376
SD.	±0.193	±0.151	±0.047	±0.234	±0.209	±0.064

When the mohair cuts of the two groups were classed it was found that the treated goats produced better quality cuts with less damage to the mohair (Table 6.6). In Table 6.6, the cuts have been ranked, in brackets, according to mohair quality (BH = 0, BFH = 1 and BSH = 2). The treated group scored 14 out of a possible 18 and the untreated control group scored only 3 out of a possible 18. This means that, in moderately to heavily infested goats, only 17 % of the goats will produce mohair of medium to good quality. It is dependent, however, on the lice loads of the individual goat. In the current study the two goats in the control group, which produced medium to good mohair cuts, were not as heavily infested with lice as the rest, who produced low-grade cuts. The one goat in the treated group which produced a low-grade mohair cut, was noted to be afflicted by a gastric condition and this might have contributed to the poor quality of the cut produced.

Table 6.5. Analysis of variance on the influence of *Damalinia limbata* on mohair mass gain.

Source of Variation	Df	SS	MS	F	P	F crit
Treatments	1	0.331	0.331	105.329	<0.001	4.494
Error	16	0.050	0.003			
Total (Corr.)	17	0.381				

Table 6.6. The results for the classing of mohair cuts on 24-8-95.

Quality	Poor	Medium	Good
Mohair class	BH	BFH	BSH
Treated mohair	1(0)	2(2)	6(12)
Control mohair	7(0)	1(1)	1(2)

Although *D. limbata* caused a loss of about 12 % in mohair production, the individual mohair loss per goat was as high as 25-26% because of lice itch. This loss of mohair production represents an average loss of income in the region of R314 per 100 kilograms or R6,28 per goat. With a loss of 25-26% of mohair production the financial loss could be as high as R13,62 per goat (calculated according to prices on 14-10-1996, which was R26.20 per kg.). This clearly represents a considerable loss of income for the farmer and therefore justifies control of the lice. In contrast, Kettle & Pearce (1974) found greasy wool production in lousy sheep to be the same. However, Kettle & Lukies (1982b)

found greasy wool production in lousy sheep to be higher than treated sheep, but also states that there may be a depressing effect, since the louse-free group were plunge-dipped a month before shearing. Wilkinson *et al.* (1982) found that lice do cause a decrease in wool production and contends that Kettle & Pearce (1974) might have found a difference in wool production had they determined the yield of clean fleece instead of greasy wool. Anson & Bell (1971) found that *D. ovis* on sheep can cause a loss as high as one-fifth (20 %) of wool production through rubbing because of lice itch. The results of the current study, on *D. limbata* on Angora goats, are in agreement with the results obtained by Anson & Bell (1971).

Goats with moderate to heavy lice loads were found to have significantly lower mohair production and quality, compared to goats not infested with lice. The reduction in mohair production and quality was due to the lice causing irritation to the goats, leading to grooming behaviour such as scratching and biting. The grooming behaviour could cause damage to mohair and skin when mohair was removed. This is in agreement with the findings of Cleland *et al.* (1989) who found that sheep carrying a medium to heavy louse infestation has significantly reduced the value and quality of the wool. According to Niven & Pritchard (1985), more wool was skirted from the fleeces of sheep treated against lice than untreated sheep. This was because several fleeces from untreated sheep contained large amounts of coloured and knotted wool and were not skirted but were classed into the cast line. Kettle & Lukies (1982b) also found that on infested wool of Australian merinos no damage was done by lice and that the damage was the result of grooming behaviour by the host. Lice also affect the skin of sheep, causing the different layers of the uncornified epidermis to thicken (Britt *et al.*, 1985; Britt *et al.*, 1986). Loss of quality may be the result of changes in the skin structure of the sheep due to heavy lice infestations. According to Britt *et al.* (1985) and Britt *et al.* (1986) the stratum spinosum, the thin stratum corneum and the sudanophilic layer, in the uncornified epidermis, are all thicker in lice infected sheep than in louse-free sheep. This change in the skin can negatively affect the production and quality of wool in sheep (Kettle & Lukies, 1982b). The

same could also apply to Angora goats.

6.3.3. The comparison of two treatment application methods.

The adult lice loads of the three groups, which were obtained post treatment, were tested with a one-way analysis of variance to check for differences (Table 6.8.). No significant differences ($F_{2, 21}=1$; $P=0.385$), in lice burdens, were found between the three groups. The average number of adult lice per goat for the control, Backline and Tickspray groups as well as the standard deviation (SD) calculated from the analysis of variance are represented in Table 6.7.

The results can be seen for the analysis of variance done on the data. The analysis was done to determine whether there were: (a) differences between lice population one and two months after treatment; (b) whether there were differences between the control and treated groups over time; (c) whether there were differences in efficacy between the Backline and Tickspray treatments and (d) whether the treatments had a significant effect on the lice populations over time (Table 6.9.).

There was no difference in population size ($F_{1;42}=1.67$; $P=0.203$), one and two months after treatment for either the Backline or Tickspray treatments (Table 6.9.). The population sizes between the control group and the Backline treatment and the control group and the Tickspray treatment differs significantly ($F_{2;42}=58.16$; $P<0.001$). Both treatment techniques seem to be equally effective in lowering lice populations at the described dosage. There was no significant difference ($F_{1; 42}=0.03$; $P=0.874$) in treatment method effectiveness between the Tickspray group and the Backline treatment group.

Table 6.7. The average number of adult lice per goat (ξ), minimum and maximum and the standard deviation (SD) for the control and two treated groups over two months, as well as the number of goats per group

Groups	N	Avg.	Min	Max	SD
One month					
Control	4	35.750	11	69	± 24.268
Backline	10	0.100	0	1	± 0.316
Tickspray	10	0.800	0	4	± 1.317
Two months					
Control	4	22.500	19	28	± 4.041
Backline	10	0	0	0	± 0
Tickspray	10	0	0	0	± 0
Overall					
Control	4	29.125	11	69	± 2.34
Backline	10	0.400	0	1	± 1.47
Tickspray	10	0.050	0	4	± 1.47

Table 6.8. A one-way analysis of variance on the initial, pre-treatment, adult lice burdens of the Tickspray, Backline treated and control groups ($P<0.05$).

Source of Variation	df	SS	MS	F	P	F crit
Treatments	2	365.46	182.73	1.00	0.385	3.47
Error	21	3841.50	182.93			
Total	23	4206.96				

Table 6.9. A factorial analysis of variance of the data of adult lice, to determine whether there was a difference between two treatment application methods and a control as well as to determine whether the synthetic pyrethroid had a significant effect on the different louse populations over time.

Source of variance	Df	SS	MS	F	P
Month	1	80.08	80.08	1.67	0.203
Between treatments	2	5569.29	2784.65	58.16	<0.001
Backl. Vs. Tickspray	1	1.23	1.23	0.03	0.874
Ave. effect of Treat.	1	5568.07	5568.07	116.3	<0.001
Month x Treatments	2	274.292	137.15	3.14	0.053
Error	42	1832.25	43.62		
Total (Corr.)	47	7755.92			

The insecticide (WipeOut) did have a significant effect ($F_{1;\,42}$=116.3; P<0.001) on the lice populations of the two treated groups and complete eradication of adult lice from the goats was achieved eight weeks after application. The Backline as well as Tickspray treatments lowered the lice populations soon after application and maintained control for the duration of the trial. None of the three lice populations (excluding the initial counts which would naturally have an influence on the treated groups) varied significantly ($F_{2;\,42}$=3.14; P=0.053) over the trial period.

WipeOut dramatically lowered the number of lice in both the Backline and Tickspray treatments compared to the control group. The Tickspray and Backline treatments reduced the adult lice populations by 97.8% and 99.7% respectively after 4 weeks, and after 8 weeks no adults were found on either of the groups.

A one-way analysis of variance was done on the initial nymphal estimates of all three groups to check for significant differences between them (Table 6.11.). The nymphal estimates of the Backline, Tickspray and control groups were found not to be significantly different ($F_{2;\,21}$=0.765; p=0.478). The standard deviation (SD) is large for all three groups because the numbers were all estimates of 10, 50,

100 etc (i.e. all rounded numbers).

Table 6.10. The average number of nymphs per goat, minimum and maximum and the standard deviation for the control and two treated groups over two months, as well as the number of goats per group

Groups	n	Avg.	Min	Max	SD
One month					
Control	4	290	90	580	227.01
Backline	10	5	0	20	8.50
Tickspray	10	5	0	20	7.07
Two months					
Control	4	402.5			268.13
Backline	10	0	0	0	0
Tickspray	10	1	0	10	3.16
Overall					
Control	4	346.250	90	580	33.25
Backline	10	2.500	0	20	21.03
Tickspray	10	3.000	0	20	21.03

A factorial analysis of variance was done on the nymphal estimates for the two months post treatment (Table 6.12.). No significant differences were found ($F_{1;42}$=0.305; P=0.584) between the two months, which indicates that, like the adult lice, the nymphal population is reduced soon after insecticide application. The three groups (Tickspray, Backline and control) were found to be significantly different ($F_{2,42}$=44.47; P<0.001). The two application methods were found not to differ significantly ($F_{1;42}$=0.0003; P=0.987) in efficacy, at a dosage of 20 ml

WipeOut per goat. WipeOut did significantly reduce the nymphal populations ($F_{1, 42}$=88.939; P=0.001) of the two treated groups, compared to the control group. No interactions were found ($F_{2, 42}$=1.29; P=0.286) between the months and the three groups, which means that the populations stayed statistically similar from one month to the next.

Table 6.11. One-way analysis of variance of the initial, pre-treatment, estimated nymphal lice burdens of the Ticspray, Backline and control groups (P<0.05).

Source of Variation	df	SS	MS	F	P	F crit
Between Groups	2	13045.83	6522.92	0.765	0.478	3.467
Within Groups	21	179050	8526.19			
Total	23	192095.8				

It is evident from the results of the factorial analysis that there was a noticeable difference between the control and Backline treatment and between the control and Tickspray treatment, but not between the Tickspray and Backline treatment (Table 6.12).

Table 6.12. A factorial analysis of variance on the data of nymphs, to determine whether there is a difference between two treatment application methods and a control as well as to determine whether the synthetic pyrethroid had a significant effect on the different louse populations over time.

Source of variance	Df	SS	MS	F	P
Month	1	2700	2700	0.305	0.584
Between treatments	2	786617.6	393308.8	44.47	<0.001
Backl. Vs. Tickspray	1	2.5	2.5	0.0003	0.987
Ave. effect of Treat.	1	786615	783315	88.939	<0.001
Month x Treatments	2	22817.6	11408.8	1.29	0.286
Error	42	371465	8844.4		
Total (Corr.)	47	1183599.6			

Figure 6.1A. shows the average population fluctuations for the adult lice of the three groups over the study period. Figure 6.1B. represents the average estimated nymphal numbers per goat for the three groups over the study period. In both instances the two treated groups have been visibly lowered over time compared to the control group.

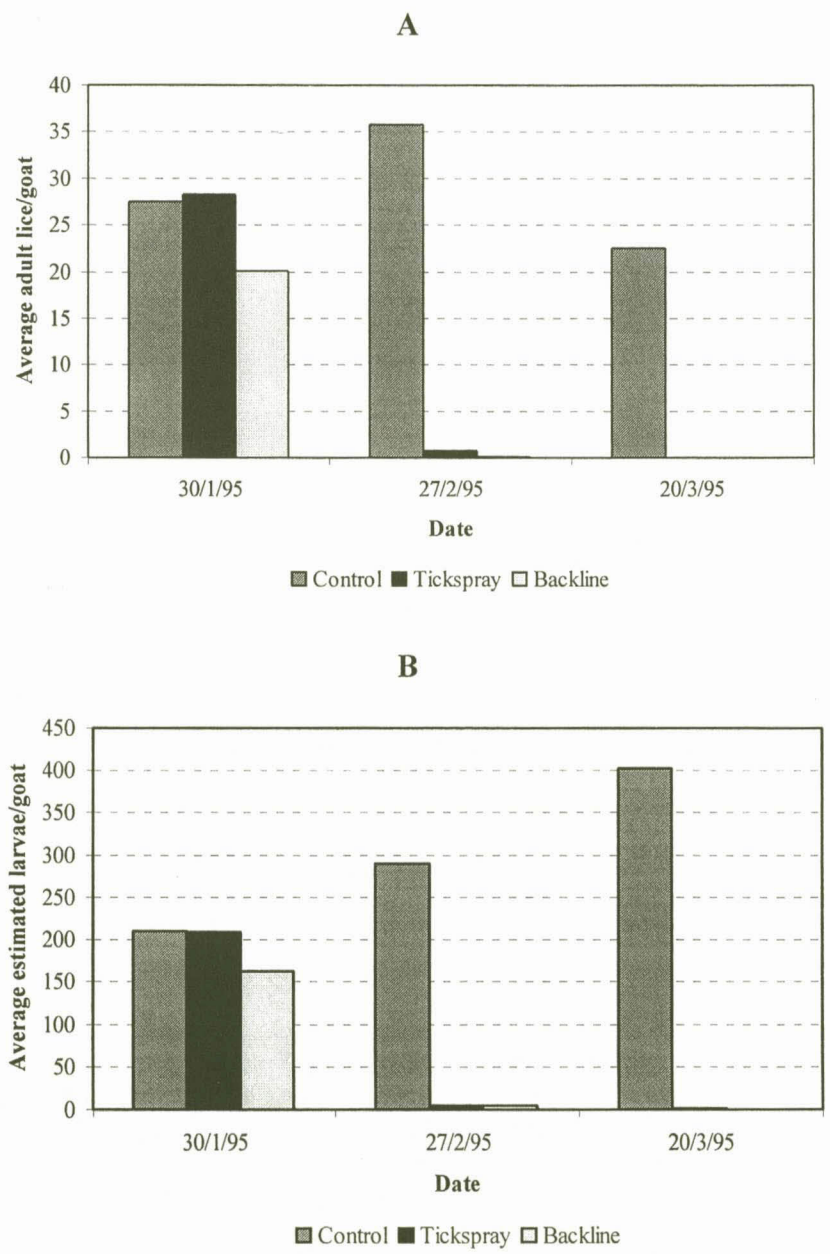

Fig. 6.1. The average number of lice per goat from the date of treatment to termination eight weeks later for the control, Tickspray and Backline groups. (A = the average number of adult and B = average estimated nymphs per goat).

During this study the different groups of goats were grazed in separate lots to reduce the transfer of insecticide or lice. Even during inspections the goats were kept apart to minimise cross contamination. Although the transfer of lice between the treated groups and the control group were minimised, nymphs still persisted on one of the goats from the Tickspray group.

Results of the current study are contradictory to results obtained during a similar study, conducted by Heath *et al.* (1992), who found that WipeOut could not eradicate lice (*D. ovis*) from sheep. This may be due to uneven distribution of the insecticide over the body of the sheep when WipeOut is used as a Backline treatment. This may lead to sub-optimal levels of chemical residue on certain areas of the body (Levot & Hughes, 1990). Synthetic pyretroid-resistant strains may be selected for in this way and may later cause control failure of the product (Levot & Hughes, 1990).

The persistence of some nymphs on the one goat in the Tickspray group does not necessarily mean that there is resistance to the synthetic pyrethroid (WipeOut). The nymphal counts are estimates and only one goat had an estimate of 10 at 8 weeks post treatment. The population size would probably be too low to constitute a serious threat of re-infestation of *D. limbata.*

The fact that WipeOut (Deltamethrin) does not completely eliminate nymphal *D. limbata* on Angora goats may suggest the prevalence of some low-level resistance to Deltamethrin. Johnson *et al.* (1992) contends that with correct calculation and application of doses, pour-on synthetic pyrethroid formulations appreciably reduces infestations of lice on all treated sheep, but failed to eradicate resistant strains of lice in 53% of laboratory trials. Kettle *et al.* (1983) found that Decacide, a Deltamethrin formulation, gave protection against *D. ovis* on sheep for about 10 weeks for off-shears and 15 weeks for treatment post shearing. Keys *et al.* (1993) also found cases where *D. ovis* showed signs of resistance to different synthetic pyrethroid formulations. Levot & Hughes (1990) contend that

sub-optimal chemical residue on certain regions of the sheep's skin may have exposed *D. ovis* on sheep, to concentrations that have selected synthetic pyrethroid-resistant types. James *et al.* (1989) showed that pyrethroid insecticide formulations, which move over the bodies of sheep, could be applied in slow release devices and reduce louse numbers to sub-optimal levels for prolonged periods. He also reported that devices that deliver high concentrations of active ingredient are less likely to select for resistance and may be able to provide both eradication and long term control of lice (*D. ovis*) on merino sheep. However, when the Backline treatment and treatment with the Tickspray applicator were compared, no significant differences were found. Because Tickspray is a lateral applicator one would expect the insecticide to be more uniformly distributed on the body of the goat. It should, therefore, be more effective in eradicating or lowering louse numbers. However, this did not happen and it would seem that WipeOut, at the rate of 20 ml per goat, has the ability to completely eradicate lice from Angora goats, even when applied dorsally like the Backline treatment.

Other sources of reinfestation of *D. ovis* could be from the introduction of new sheep that are infested with lice (Anson & Bell, 1971). This might also apply to *D. limbata* on Angora goats as they have similar behavioural patterns and life cycles. Both species spend their whole lives on their hosts where they feed on the sebum and skin scruff of their hosts. Thompson *et al.* (1994) tested the influence of rainfall on the efficacy of ivermectin jetting fluid for the prevention of fly strike and the eradication of *D. ovis* infestations on long-wooled sheep. It was found that rainfall had no influence on the efficacy of the insecticide. This factor can also be discounted in the current trial, as there was very little precipitation during the study period. Thompson *et al.* (1994) also found that long wool might negatively influence the efficacy of jetting fluids, as the fluid can not penetrate the dense wool of Australian merino sheep. For the best results it is recommended that sheep should be treated directly after shearing (Fourie *et al.* 1995; Rugg *et al.*, 1995). Long mohair is not as dense as wool and the penetration of jetting fluids are not affected to the same extent as on sheep. It is,

however, preferable to treat goats after shearing as this will lower the effect that the mohair has on the dispersal of the lousicide and allow penetration of the insecticide down to the skin where the lice are most prevalent.

The success that is achieved with lousicides can be influenced by factors outside the control of the manufacturer such as wrong application of the chemical or the operator being less than competent in operation of the equipment or might have misunderstood the instructions (Morcombe & Young, 1993). Another cause of treatment failure is resistance to synthetic pyrethroids, which has reduced the efficacy of these chemicals, particularly when applied as backline treatments, against certain strains of lice (Levot & Hughes, 1990; Johnson *et al.*, 1992; Morcombe & Young, 1993). An alternative application method, ensuring a more uniform dispersion of the insecticide is necessary. To this end, the Tickspray lateral treatment applicator was developed.

6.4. Conclusion.

It was found that *D. limbata* does not affect the body mass of Angora goats to any significant degree, but may rather be an indicator of other diseases the goats are suffering from, such as gastric afflictions. *D. limbata* does, however, have a significant and negative influence on mohair production and quality and can cause substantial financial losses to the farmer.

Because these lice have such a negative effect on mohair production and quality an efficient and effective means of lice control is indispensable. The two application methods did not show a significant difference in efficacy, as far as delivering the lousicide at high enough concentrations, to eradicate lice all over the body of the goat. The Tickspray applicator is, however, more efficient as far as labour and time consumption is concerned. At lower concentrations of the lousicide the Tickspray applicator would probably give a more even distribution of chemical on the body of the goat, reducing the chances of getting areas with

sub-optimal doses of the chemical.

CHAPTER 7.

GENERAL CONCLUSION.

Prior to the middle of the 1900s biting lice were not viewed as economically important ecto-parasites of domesticated animals. They were seen as a nuisance and no more. However, more critical study of especially *Damalinia ovis* on sheep, but also other species, showed that these lice can impact greatly on the economic output of the host animal involved. With the high input costs in agricultural practices today the return must justify the costs incurred. For these reasons any factors that influence the product negatively must be minimized. To accomplish this it is important for the producers to have an intimate knowledge of these factors.

Mohair producers in South Africa are being hampered in their efforts to manage *D. limbata* on Angora goats because of this lack of knowledge. During the current study, various aspects of the bio-ecology of these lice were investigated.

The micro-habitat of *D. limbata*, close to the skin of the goat, stayed relatively constant at a temperature of approximately 35 °C. This constant environment can, however, be severely disrupted by shearing which, in turn, can provide the farmer with an opportunity for more effective control of lice.

The establishment of a sustainable *in vitro* colony could not be achieved due to quality problems experienced with the artificial medium used. To ensure the vitality of an *in vitro* colony it is important to obtain skin squares which are fresh when they are prepared.

By using head capsule measurements (head width and head length) it was possible to determine that *D. limbata* has tree nymphal instars before adulthood. These results, however, are solely based on field collected lice and should be validated using laboratory reared lice for greater precision. Unfortunately this could not be done during this study.

When Angora goats have long hair the lice are well sheltered from environmental influences such as solar radiation and low or high environmental temperatures. However, when the goats are shorn, as was the case during July and February, the lice are exposed to widely varying environmental influences. This is especially true during winter when the minimum and maximum environmental temperatures, on any given day, can vary by as much as 20 °C. If one includes the lice removed during shearing, the resultant decrease in lice populations can be drastic.

There was movement of the lice populations over the body of the goat during the different seasons. Adult populations seemed to favor the ventro-lateral areas of the body while the nymphal populations were more abundant on the dorso-lateral areas of the body.

When attempting to manage *D. limbata*, these factors can be exploited to greatly enhance the efficacy of control. The application of chemical control directly after shearing, when lice populations are most vulnerable, will also have the added advantage of better penetration and spread of the insecticide being applied. If control during the winter is good, when the lice populations are at its lowest, the lice populations would take much longer to recover and would have a much smaller impact during the summer.

During the current study, it was found that *D. limbata* does not have a negative influence on the body mass of Angora goats. *D. limbata* does, however have a

significantly negative effect on the mohair production of Angora goats. Significantly less mohair were produced by goats with moderate to heavy lice loads. Mohair quality is also greatly reduced when the goats have moderate to high lice loads. This can result in a substantial loss of income for the farmer. For the effective control of any species of pest, an effective means of delivering the control agent is indispensable.

The two application methods, which were used to deliver WipeOut, showed no significant differences in efficacy at the recommended dosage and eradicated lice effectively on all parts of the body. The lateral applicator, Tickspray, was, however, more efficient as far as labour and time consumption was concerned. At lower concentrations of the lousicide the Tickspray applicator would probably ensure a more even distribution of the chemical over the body of the goat. This would, in turn, reduce the chances of finding areas with sub-optimal levels of the chemical.

Further studies should include (1) a more detailed study of the biology of these lice. Especially the areas pertaining to embryo and nymphal development. (2) Head capsule measurements of field populations should be compared to laboratory reared lice to validate accuracy. (3) To this end a viable and sustainable *in vitro* colony is essential. (4) Bio-assays should also be done, on the lice populations, to determine resistance levels within the populations to the various chemicals used in combating these lice.

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