ASPECTS OF THE BIOLOGY, SPECIFIC STATUS AND CONTROL OF THE BEDBUGS *Cimex lectularius* AND *Cimex hemipterus* IN NORTHERN NATAL AND KWAZULU

by

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Eshowe

1989

ABSTRACT

The bedbugs Cimex lectularius and C. hemipterus (Hemiptera: Cimicidae) have been identified from a study area in northern Natal and KwaZulu, South Africa, between 26° 45′ S and 28° 45′ S, and 32° 00′ E and 32° 52′ E. Morphological behavioural data suggest that the pigeon bug, C. columbarius, which closely resembles C. lectularius, is not present in Zulu huts. Two characters are suggested to supplement Usinger's (1966) key for distinguishing the nymphal stages of C. lectularius, and a key is presented for the case of C. hemipterus. Distinguishing features between the nymphal stages of the two species are also given. The two species were found to be sympatric over most of the study area, with C. hemipterus the more plentiful species in the north and apparently still in the process of invading the study area. Evidence was found that C. hemipterus replaces C. lectularius in huts where initially both species were found, and this agrees with laboratory findings that interspecific mating tends to shorten the life span and reduce the egg production of female C. lectularius females. Interspecifically mated female C. lectularius can be recognised by an easily visible morphological change in the reproductive system (Walpole, 1988b) and this character was used to prove that interspecific mating takes place in the wild.

The size and life-stage composition of bedbug infestations were investigated together with factors likely to affect their growth. Cimex hemipterus was found not to be at a

disadvantage as regards rate of egg production despite low temperatures in the wild, which suggested that such interspecific differences apparent in the laboratory may be an artifact. The specific status of C. lectularius and C. hemipterus are discussed, with particular reference to their cross-mating in the wild and the production of a hybrid. The acceptability of the Recognition Concept of species (Paterson, 1985) is considered in detail. The susceptibilities of wild bedbugs of both species to DDT, dieldrin and fenitrothion were tested in comparison with a susceptible strain of C. lectularius. Field trials of bendiocarb, deltamethrin and fenitrothion were carried out involving monitoring by bioassay and insecticidal knockdown. Subsequent to successful bedbug control operations, reinfestation rates and rates of replastering of mud walls of huts (an indigenous method of attempted bedbug control inimicable to malaria control operations) are assessed.

PREFACE

The work described in this thesis was completed while I was a part-time student in the Department of Zoology and Entomology under the supervision of Dr. C.C. Appleton.

These studies represent original work by the author and have not been submitted in any form to another university. Assistance given to me during field work has been duly acknowledged.

KEITH NEWBERRY

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I am most grateful for the friendly and helpful guidance of the Electron Microscope Unit staff of the University of Natal, Pietermaritzburg, namely Mr. A.G. Bruton, Mr. V. Bandu, Mrs. B. White and Mrs. P. Donelly. This study would not have been possible without the assistance of the staff of the National Institute for Tropical Diseases at Eshowe and Jozini during the field work. I thank Messrs. E.J. Jansen, Z.M. Mchunu, A.G. Quann, S.Q. Cebekhulu, H.M. Makwhaza and G.R. Thibaud for their assistance in supervising collection of bedbugs, and for their help in the insecticide trials. With the exception of the electron microscope studies and a Medical Research Council capital grant awarded to me in 1987, the work was funded by the National Institute for Tropical Diseases.

At the height of the laboratory phase of this work, Mrs. V. Worthington assisted in the basic tasks of processing field collections ready for scoring, and fed the colonies when I was on leave for two months. Her help is much appreciated, especially in the absence of any laboratory staff, and in view of the fact that she holds an administrative post.

I should like to thank Mr. A.J. Pope of Coopers (SA) (Pty) Ltd., for the deltamethrin and fenitrothion used on field trials, the technical grade insecticides used in susceptibility tests, and the Insectigas which enabled effective monitoring of insecticide trials. I am also grateful to the South African Bureau of Standards, Pretoria, for sending me bugs from their colony.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 THE FAMILY CIMICIDAE AND THE BEDBUGS

The Cimicidae (Hemiptera) are blood-sucking, ectoparasitic bugs which are dorso-ventrally flattened, between four and seven millimetres long, and remain near the resting places of their hosts when not feeding rather than on the hosts themselves. Only three of the seventy four species of Cimicidae prey on man (Table 1), the rest being associated with bats and birds (Usinger, 1966). With the exception of two species, all Cimicidae pass through five nymphal stages before attaining adulthood (Usinger, 1966) (Plates 1 and 2).

Table 1. Taxonomic classification of the three anthropophilic Cimicidae (Usinger, 1966)

Family:	Cimicidae	
Subfamily:	Cimicinae	Cacodminae
Genus:	Cimex	Leptocimex
Species:	lectularius Linnaeus	boueti Brumpt
	hemipterus (Fabricius)	

Haeselbarth et al. (1966) gave details of four South African Cimicidae, all of which were associated with bats. Cacodmus sparsilis was described from one female bug taken from a Scotophilus nigritus in Durban, and has also been recorded from Pietermartizburg, hosts unknown. Cacodmus villosus

Plate 1. Life-stages of C. lectularius, the Common Bedbug, showing differences in size between the egg, the five nymphal stages and the male and female adults. Magnification 20x.

M A L E

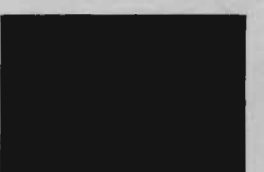
F E M A L E

5th STAGE NYMPH

4th STAGE NYMPH

3rd STAGE NYMPH

2nd STAGE NYMPH



1st STAGE NYMPH

EGG

Plate 2. Life-stages of C. hemipterus, the Tropical Bedbug, showing differences in size between the egg, the five nymphal stages and the male and female adults. Magnification 20x.

M A L E

F E M A L E

5th STAGE NYMPH

4th STAGE NYMPH

3rd STAGE NYMPH

2nd STAGE NYMPH

1st STAGE NYMPH

EGG

has been found in Lichtenburg and Johannesburg on Eptesicus capensis, with Pipistrellus musciculus being a host in the Congo. Stricticimex antennatus was collected from Bredasdorp and Three Sisters' Rocks in Cape Province, without host records. Stricticimex transversus is recorded from Bloemfontein, and is known to use Scotophilus nigritus and Tadarida bocagei as hosts in South West Africa/Namibia and Botswana respectively. Overal & Wingate (1976) observed S. antennatus feeding on Myotis tricolor, Rhinolophus simulator, R. clivosus, Nycteris thebacia, and Hipposideros caffer as well as human visitors to a cave near Pietermaritzburg. Usinger (1966) listed another South African batbug, Aphrania barys, from Westminster in the Orange Free State, which has been found on Eptesicus zuluensis in the Namib Desert. Ledger & Kritzinger (1974) described the first avian bug from sub-Sarahan Africa Paracimex africanus, from the nests of the swifts Tachymarptis melba africanus and T. aequatorialis gelidus in Pietersburg district and Zimbabwe respectively.

A specimen of Afrocimex, as yet unidentified to species, has been found in a cave near Potgietersrus (A J Cornel, pers. comm.*), and there are at least two, possibly four, Cacodmus species and one Aphrania species new to science

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discovered along with Stricticimex intermedius in the Kruger National Park (L E O Braack, pers. comm.**).

Apart from the location of one or two capture sites, host species, and a morphological description, nothing is known of all but one of the zoophilic South African Cimicidae. The exception is Stricticimex antennatus, and the findings concerning the biology of this batbug (Overal & Wingate, 1976) are considered in the appropriate sections of this thesis. The distributions of the two anthropophilic bedbugs, Cimex lectularius and C. hemipterus are discussed below and in detail in Chapter Three

Some Cimicidae do not attack man even when their hosts dwell in human habitations and the opportunity arises. In Europe Cimex columbarius infests lofts frequented by pigeons (Ueshima, 1964) but has never been reported biting man. Hertig (cited by Usinger, 1966) related that in China C. flavifusca seen crawling around walls did not bite the human occupants of the house. Spencer (1935) observed a similar situation involving another batbug, C. pilosellus, in North America. Other Cimicidae will bite man if he enters their environment. Men chopping down palm tree leaves frequented by bats were badly bitten by Aphrania vishnou in India (Mathur, 1953), and people entering a cave used by bats were

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fed on by Stricticimex antennatus near Pietermaritzburg (Overal & Wingate, 1976).

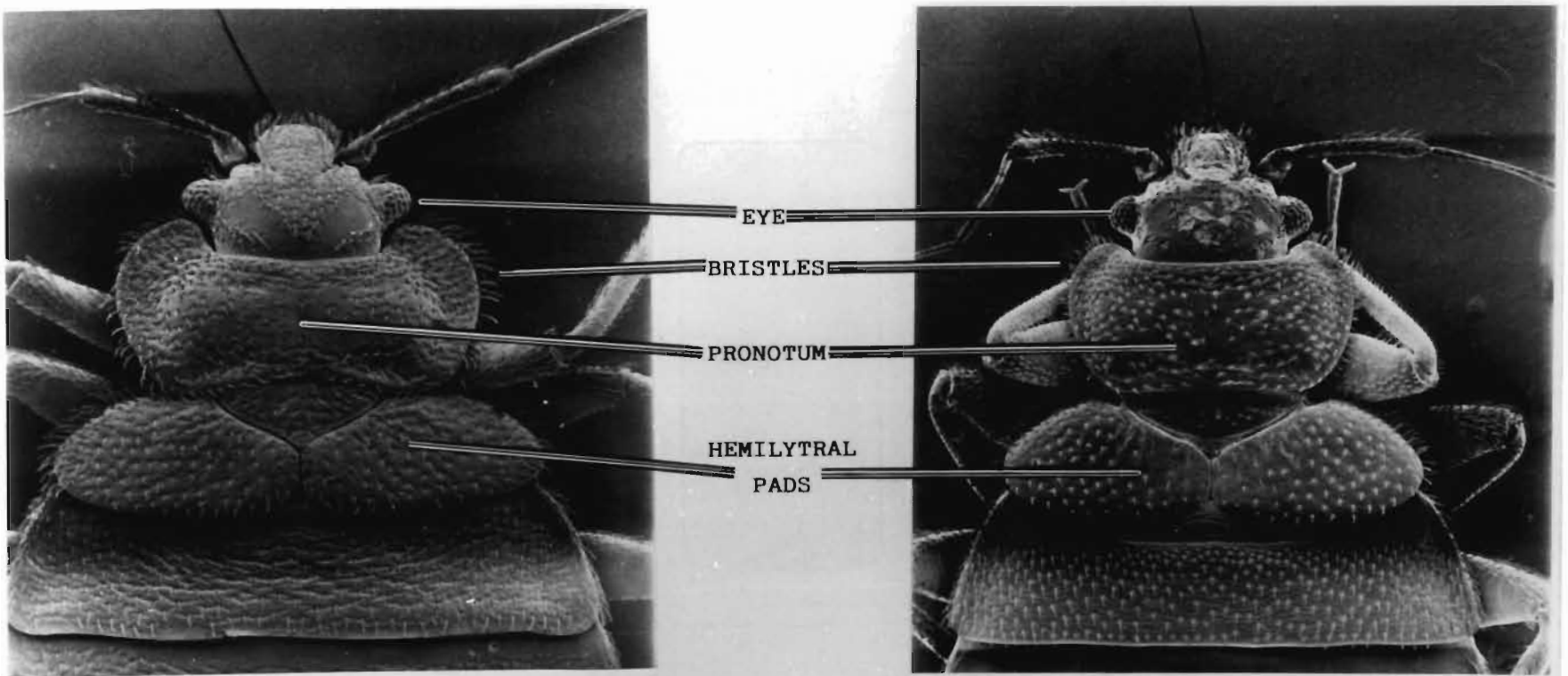
The three anthropophilic species, Leptocimex boueti, Cimex lectularius and C. hemipterus are termed bedbugs because they infest human habitations and usually feed on man during the night when he is asleep. The bedbugs do not occasionally bite man under unusual circumstances, as is the case with other Cimicidae, but have adopted man as a major host. Cimex lectularius is believed to have evolved in association with bats, perhaps first becoming involved with man in palaeolithic times when he dwelt in caves. Bats remain an important host, supplemented by pigeons, sparrows and swallows (Hase, and Kemper, cited by Johnson, 1941), chickens (Usinger, 1966) and laboratory rats (Mellanby, 1939). Cimex hemipterus thrives on poultry farms (Rosen et al., 1987) and has also been found feeding on bats, but this species is mainly a parasite of man (Usinger, 1966).

Leptocimex boueti has been recorded from bat caves and human habitations in the Sudan and West Africa (Usinger, 1966). Readily distinguishable from the other two bedbugs by its smaller size, long legs, and very elongated third antennal segment, L. boueti has never been found in South Africa. Cimex lectularius, the Common Bedbug, has a virtually world-wide distribution (Usinger, 1966) though it is absent from some areas of the Orient. Omori (1939) suggested that the adverse effects of interspecific mating with

C. hemipterus could account for this restriction in range (see Chapter Six). Cimex hemipterus, the Tropical Bedbug, is found throughout the tropics, or just outside them, there being one record from Natal (Usinger, 1966) and a recent report from Israel (Rosen et al., 1987). Cimex lectularius and C. hemipterus are easily distinguishable in the adult stage by the much broader pronotum of the former species (Plate 3). Other differences are apparent at 10x magnification including the rounder hemelytral pads and more developed eyes of C. hemipterus. Male C. hemipterus have a distinctly convex shape to the rear of the right side of the abdomen (Plates 1 and 2, pages 2 and 3). The bristles of C. lectularius curve backwards, as compared with the straighter bristles of C. hemipterus which are set more perpendicular to the integument.

Cimex lectularius is extremely similar morphologically to the pigeon bug, C. columbarius. The two species have different means for the ratio of head-width to third antennal segment length, but their ranges for this character overlap so identification can be carried out on populations but not with certainty on individuals (Johnson, 1939). Four bedbugs intermediate in this character were collected at Kokstad, South Africa (Johnson, 1939), but the presence of C. columbarius has not otherwise been suspected outside Europe (Usinger, 1966).

Plate 3. *Cimex lectularius* and *C. hemipterus* males showing interspecific adult differences, the former species having less ocelli at the base of the eye, a wider pronotum, less rounded hemelytral pads, and more recurved bristles.



Cimex lectularius

Male

head and thorax

0,5 mm

Cimex hemipterus

Male

head and thorax

0,5 mm

1.2 PHYSIOLOGICAL COMPARISONS BETWEEN THE BEDBUGS

The dissimilar geographical distributions of C. lectularius and C. hemipterus prompted laboratory investigations aimed at revealing differences in the reactions of two species to temperature and humidity. The comparative studies of Hase (1930) and Mellanby (1935) suggested a greater physiological adaptation to temperate conditions by C. lectularius, a finding which was confirmed by the repetition and extension of this work by Omori (1941) in a 174 page treatise which is briefly summarized in the following paragraphs. Generally, C. lectularius functioned best between 18 and 30 degrees C, and C. hemipterus between 22 and 33 degrees C, with C. lectularius the more prolific at all but very high temperatures over 33 degrees C.

At all temperatures tested, C. lectularius had the longer preoviposition (insemination to egg laying) and egg laying periods, and briefer durations of nymphal stages. Below fluctuating temperatures about a mean of 18 degrees C, C. hemipterus females were able to lay fewer than six eggs per month which hatched only when the mean temperature was above 20 degrees C. At constant temperatures, 0,47 eggs per day were laid at 18 degrees C by C. hemipterus, though only 15% hatched. In contrast, C. lectularius laid 0,64 eggs per day at 15 degrees C with a 70% hatching rate. Both species produced their maximum average number of eggs (5,40 and 6,84) and surviving nymphs (4,74 and 6,41) per day at 30

degrees C, with a sharp drop off at lower and higher temperatures for C. hemipterus and C. lectularius respectively. Eighty one per cent of the eggs of the latter species died at 33 degrees C, with a mortality of 38% among hatched nymphs, while only 6% of C. hemipterus eggs failed to hatch and nymphal mortality was 57%. At 36 degrees C the successful completion of the life cycle was severly stressed in C. hemipterus but broke down completely in C. lectularius.

High humidity (98% r.h.) and high temperature (33 degrees C) delayed the rate of development of C. hemipterus third, fourth and fifth stage nymphs, but not C. lectularius, and caused slight mortality to nymphs of both species. High humidity and temperature was also capable of reducing the longevity of C. lectularius adults by 47 to 61% and C. hemipterus by up to 31%, compared to survival at 71% r.h.

Omori (1941) stated that starving C. lectularius always live longer than C. hemipterus. His data show this is generally the case, though not at 27 degrees C when females of these species lived an average of 41 and 43 days respectively. Starving specimens of C. lectularius live a very long time, well over a year, at 10 degrees C, though survival times below this temperature are erratic. Longevity of starving C. hemipterus is about a year at 10 degrees C, but falls to 80 days at 6 degrees C compared to 280 days for C. lectularius. Although adult bugs of the two species mix randomly when

placed on a surface of uniform temperature, they segregate into clusters at 28 to 29 degrees C (C. lectularius) and 32 to 33 degrees C (C. hemipterus) on a graded temperature plate (Omori, 1941). This again underlies the trend that the metabolisms of the two species function optimally at mean temperatures three or four degrees apart.

1.3 THE MEDICAL IMPORTANCE OF BEDBUGS

Bedbugs feed by pushing the stylets deep into the sub-cutaneous tissue of the host's skin until a blood vessel of suitable size is penetrated. Saliva injected early in the feeding process is the cause of allergic reactions experienced by many, but not all, of hosts bitten (Usinger, 1966). Some people become desensitized after receiving many bedbug bites over a long period, but others do not and for them spending a night in an infested room is a painful and debilitating experience. The skin may be broken by scratching the itchy erythematous papules caused by the bites, and secondary infection can then occur. Iron and haemoglobin levels in the blood are depleted when many bugs feed, Usinger (1966) finding his own haemoglobin down to 11,5 g per 10 ml of blood from 14,5 g after five years of feeding bugs on himself, despite iron supplementation.

Cimicidae present an obvious danger as vectors of disease organisms which are able to live, and perhaps multiply, in the bedbugs. Burton (1963) listed 30 human diseases which

are capable of surviving in bedbugs for varying lengths of time, but the ability of bedbugs to transmit these diseases in infectious form to man has not yet been conclusively demonstrated. However, bugs have been shown to act as vectors of animal diseases. Gardner and Molyneux (1988) found Trypanosoma incertum in 33 (16%) of 206 Pipistrellus pipistrellus bat bugs caught at various sites in Britain. The trypanosome developed and multiplied in P. pipistrellus and in C. lectularius when infected in the laboratory, and the organism was transmitted to previously uninfected bats when the bugs fed on them. Paterson et al. (1984) found the bat parasites Trypanosoma myoti, T. hendricki and T. vespertilionis multiplied and developed in C. lectularius, with the latter two species being transmitted by the bug to uninfected bats. Zazara et al. (1986) found the chicken disease organisms Salmonella gallinorum-pullorum and Pasteurella multocida could exist in C. lectularius for 17 days and 24 hours respectively, though transmission of these organisms by the insect to susceptible birds was not achieved. Eley et al. (1987) isolated a reovirus from C. lectularius, and Williams et al. (1976) found Kaeng Khoi virus in wild bugs Stricticimex parus and Cimex insuetus as well as the bat Tadarida plicata which suggests the bugs may be vectors.

The most convincing, though not quite conclusive, evidence that bedbugs act as vectors of human disease comes from investigations concerning hepatitis B. Jupp et al. (1978)

working on C. lectularius in the northern Transvaal tested pools of ten bugs for the presence of hepatitis B surface antigen (HBsAG) as an indication of the presence of the complete virus. Estimated infection rates of C. lectularius with hepatitis B virus ranged from 17,1 to 67,0 per 1000 bugs, with engorged and unengorged insects having infection rates of 34,8 and 25,3 respectively. These authors suggested that the very high rates of infection, especially in unengorged bugs, indicated that C. lectularius could be a vector of this virus in the Transvaal. As bedbug infestations are far more often found in houses used by the Black population, this could account for the especially high prevalence of hepatitis B in this group.

Jupp et al. (1979) concluded that hepatitis B virus remained in C. lectularius for over 7 1/2 weeks, could be transmitted trans-stadially over one moult only, but not transovarially, and did not multiply within the bug. Antibody to HBsAG was found in a rabbit and guinea pigs on which HBsAG-positive bugs had fed, suggesting the mechanical transmission of hepatitis B virus. The absence of biological multiplication, and hence biological transmission, was confirmed by Jupp et al. (1980) and Jupp et al. (1983). This contradicts Taylor & Morris (1980) who read their data as indicating an increasing proportion of bugs showing positive 2 - 3 months after infection with hepatitis B virus, and interpreted this as viral replication. However, the proportions of HBsAG-negative to HBsAG-positive bugs 3 to 52 days and 59 to

94 days after infection (their Table 2) are not significantly different (chi-squared = 2,3 for 1 degree of freedom) even without the loss of a degree of freedom for the retrospective selection of data for comparison. These authors' findings that C. lectularius remained HBsAG positive for 17 1/2 weeks, and from first instar to adult, stand in contrast to the results of Jupp and colleagues mentioned above. Cimex hemipterus is also suspected as an agent of hepatitis B transmission. Wills et al. (1977) found infection rates of 22% (n = 55) and 47% (n = 17) in unengorged and engorged C. hemipterus from rural Senegal, and Ogston et al. (1979) found HBsAG persisted for up to six weeks in C. hemipterus which had taken several HBsAG-negative blood meals since infection.

Jupp et al. (1983) used the detection of the antigen HBeAG as indicative of the presence of infective virus, and found both HBeAG and HBsAG in the faeces of C. lectularius fed on blood containing hepatitis B virus. This contradicted the negative finding of Newkirk et al. (1975). Jupp et al. (1983) concluded that C. lectularius could infect man if an engorged bug were squashed on to broken skin, or via infected faeces, or if bugs regurgitated during feeding. A bug moving from one host to another after a partial feed could possibly transmit the virus when probing for a second blood meal. Ogston & London (1980) found C. hemipterus also excreted HBsAG, but since neither direct nor indirect tests for infectivity were carried out, it has not yet been

demonstrated that C. hemipterus can mechanically transmit infective hepatitis B virus.

Lyons et al. (1986) showed that the human immunodeficiency virus (HIV) could survive for one hour in C. lectularius. A later study by Jupp & Lyons (1987) proved HIV was still alive four hours after C. lectularius had fed on infected blood, and two hours after C. hemipterus had ingested the virus. These authors concluded, however, that the low infectivity of HIV and the short survival time of the virus in bedbugs meant that these insects are not important in the spread of acquired immunodeficiency syndrome (AIDS) in man.

1.4 RESEARCH ON THE BEDBUGS OF NORTHERN NATAL AND KWAZULU

1.4.1 Introduction

During 1980 the Health Department of Kwazulu received many complaints about bedbugs from the inhabitants of the northern coastal zone stretching from the Mozambique border (26^{o} 45′ S) down to 28^{o} 15′ S, and east of 32^{o} E (see Fig. 1 of Newberry et al. (1987), page 53 of this thesis). The area, though south of the tropics, has a tropical climate with a mean winter temperature of over 18 degrees C (Poynton, 1964).

In this rural region, black people mainly of the Zulu tribe mostly live in single room huts with mud walls and thatched

or corrugated iron roofs. Dwellings with concrete walls are becoming increasingly evident, as are houses with more than one room, but such structures are still very much in the minority.

At the time I was requested to investigate the bedbug problem, nothing was known beyond the identification of some specimens collected from the area as C. lectularius. A broadly-based study was therefore carried out to investigate the biology of bedbugs in the affected region, and bring about their control. Bedbugs were collected from all over the study area to determine which species were present. Morphological criteria given in Usinger (1966) were used to identify adult specimens to species level and to distinguish between the nymphal stages of C. lectularius. Characters had to be found to identify the nymphal stages of C. hemipterus, and to separate the nymphal stages of one species from the other (Chapter Two). Because there is no gene flow between the two bedbug species (Omori, 1939), their capacities to become vectors of diseases, or to develop resistance to insecticides, must be regarded as separate and potentially different. A knowledge of the distribution of the two species in the study area was therefore important (Chapter Three).

All previous studies in the literature assume that laboratory observations will lead to accurate insight concerning the comparative ability of the two bedbug species

to survive in the wild. The ability of each species to adapt equally to laboratory conditions of permanent confinement and arbitrarily spaced blood meals taken from hosts not encountered in the wild, has never been questioned. One physiological process, the production of eggs, was therefore studied for both species in the laboratory and under conditions where bugs lived freely until egg laying. A comparison of laboratory and field results concerning a vital part of the reproductive cycle was then made (Chapter Four).

The impact of a bedbug infestation in terms of irritation from bites and transmission of disease depends both on the size of the infestation and life-stage composition of the population at the time. Bugs in early nymphal stages inject less irritant saliva, remove smaller volumes of blood, and have been exposed less often to human diseases they might transmit, than have later stage nymphs and adults. The size and life-stage composition of bedbug infestations were therefore studied and considered in relation to the availability of hosts and the efforts made by hut occupants to rid their homes of the insects (Chapter Five).

In the laboratory, mating between male C. hemipterus and female C. lectularius reduced the lifespan and production of viable eggs of the latter, whilst the reciprocal cross had no adverse effects on female C. hemipterus (Omori, 1939). Omori went on to suggest that C. hemipterus prevented

C. lectularius from establishing itself in areas of the Orient because of the deleterious effects it suffered after interspecific mating. This hypothesis is far more controversial today, in the light of recent species concepts (Paterson, 1985) than when it was first proposed. Competitive interaction between two pest species could also be of practical importance, so this phenomenon was investigated further (Chapter Six). The results of this research raised doubts about the acceptability of the Recognition Concept of species (Paterson, 1985), and posed the question of whether the two bedbug types should be regarded as two distinct species, subspecies (Walpole, 1988a), or as belonging to the same species without subdivision. This very basic taxonomic problem is considered in Chapter Seven.

Although all huts in the study area are sprayed annually with DDT for malaria control purposes, bedbugs still constituted a problem. The effects of DDT on bedbug infestations were therefore investigated. The susceptibilities of local bugs were determined as regards DDT and other insecticides which were subsequently tested for use in large scale bedbug control operations. Bedbug control would not be practicable if the insects returned to treated dwellings as soon as the brief residual life of the insecticide used was over. The reinfestation studies carried out gave information about whether the insecticidal control of bedbugs in the area was worthwhile, and in addition

provided data on the efficiency of bedbug dispersal. Surveys were also carried out to ascertain whether the eradication of bedbug infestation reduced the rate of replastering of mud walls by hut occupants. (One of the reasons for replastering is to trap bedbugs in cracks in mud walls. This process also covers DDT deposits sprayed for malaria control purposes, however, and is therefore a cause for concern for the Health Department) (Chapter Eight).

All the above work is reviewed in Chapter Nine.

1.4.2 Materials and Methods

Laboratory colonies

Cimex lectularius and C. hemipterus collected from Zulu huts in the study area were kept in plastic honey jars 122 x 60 mm each containing a cardboard rectangle reaching from the base of the jar to the lid. The screw-on lids were modified by removing the centres and inserting mosquito netting through which the bugs could feed. Cimex lectularius from the colony maintained at the South African Bureau of Standards (SABS) in Pretoria were also kept, and the three colonies were fed once or twice a week, occasionally after two weeks, as described below. The insects were kept in a heated insectary with an average temperature of 25 degrees C and ambient relative humidity ranging between 38 and 85%.

Colonies were initially cleared of exuviae every few weeks, but it was found subsequently that the insects flourished best without any disturbance. The SABS C. lectularius thrived over three years without the need for supplementation. The colonies founded by wild-caught bedbugs of both species were frequently augmented by fresh material from the field, but even so often went to extinction. Eventually self-sustaining colonies of both species were obtained, though C. hemipterus never attained the numbers of SABS or wild C. lectularius.

The feeding of bedbugs in the laboratory

Guinea pigs (Cavia cobaya) were injected intraperitoneally with Sagatal, pentabarbitone sodium, at a dosage of 0,5 ml Sagatal per kg body weight, each injection being diluted to 2 ml with injectible water. Sedation occurred between 15 and 60 minutes after treatment, when the guinea pigs were shaved using electric hair clippers, and lain on their sides on mosquito netting tops of jars containing bugs. Other bug containers were held by retort stands and applied to the upper side of each guinea pig.

Scanning electron microscopy

Cimex specimens were stored in 70% alcohol, and were immersed in 80% then 90% for ten minutes at each concentration before being put in absolute alcohol for

twenty minutes with one change. Specimens were then critical point dried by a Hitachi HCP 2. Bugs were mounted on brass stubs using double-sided tape, and coated with gold palladium by a Polaron ES 100 sputter coater. Micrographs were taken with a Hitachi S 570 Scanning Electron Microscope, initially operating at 5 k v. Some photographs were marred by charging believed to be caused by the double-sided tape or inadequate coating of the specimens. When this occurred, re-coated specimens were photographed again at 3 k v. Other methods of mounting using colloidal graphite or silver dag were not possible due to the small size of the material.

Further details of materials and methods are given in the relevant sections of this thesis. Papers which are published and in press included in the text of this thesis have their own tables, figures, acknowledgements and references.

CHAPTER TWO

THE IDENTIFICATION OF BEDBUG SPECIES AND THEIR LIFE STAGES

2.1 INTRODUCTION

The Cimicidae found in human dwellings in Natal and KwaZulu can be allocated to the subfamily Cimicinae because in all cases the paragenital sinus is ventral; lateral pronotal bristles are serrated on the convex sides or at obliquely truncated tips, and the metasternum forms a flat plate between the coxae. These domestic insects belong to the genus Cimex because there is no subapical row of short, stout spines on front and middle femora; the ectospermalege is a transverse thickening of the interior margin of the sixth ventrite; hemelytral pads have convex hind margins; the second antennal segment is subequal to the interocular space, and the pronotum is 1,5 or more times, but less than twice, as wide as the head. The taxonomic characters of a narrow cleft paragenital sinus with bristles around it; hind femora more than 2,6 times as long as wide, and hind margins of hemelytral pads broadly rounded on inner halves, indicate the bugs belong to the Hemipterus and/or Lectularius groups. Those adult bugs found in this study which have the pronotum less than 2,5 times as wide as long in the middle were classified as C. hemipterus, whilst those in which the pronotum is more than 2,5 times as wide as long belong to the Lectularius group (Usinger, 1966) (Plate 3, page 8).

2.2 THE IDENTIFICATION OF THE LECTULARIUS GROUP SPECIES PRESENT IN SOUTH AFRICA

2.2.1 Introduction

Cimex lectularius infests human habitations and feeds on man, but is also found in association with other hosts, including wild and domestic birds (Chapter One). Jenyns (1839) described a morphologically very similar bug, C. columbarius, found only in association with birds, particularly pigeons. So slight are the differences between the two bug species that identification tended to be influenced by the probable host, with insects found on pigeons assumed to be C. columbarius, and those in houses designated as C. lectularius (Johnson, 1939). Some C. lectularius were therefore certain to have been misidentified as C. columbarius, though the reverse error is less likely.

Jenyns' (1839) description of C. columbarius consisted of subtle morphological comparisons with C. lectularius, with only the ratio of the 3rd/4th antennal segment lengths being easily quantifiable. Whilst investigating the validity of characters to separate individuals or samples of the two species, Johnson (1939) was aware of the unsatisfactory basis of his endeavour. He was trying to find a morphological trait to distinguish between two species which could not *a priori* be reliably separated on other grounds.

However, he justified his approach retrospectively in that the method he developed led to identifications which accorded with expectations from host association. Separation of C. lectularius and C. columbarius is possible by cytological means only in areas where they have different karyotypes. Cimex columbarius always has 26 autosomes plus X1X2Y in the male and X1X1X2X2 in the female. Cimex lectularius may have the above karyotype or a diploid number between 29 and 36 for the male and 30 to 42 for the female, dependent on the number of X chromosomes present or, rarely, an extra pair of homologous autosomes (Ueshima, 1966).

Following Usinger (1966), European C. lectularius usually has six X chromosomes and can therefore be distinguished from C. columbarius, but in South Africa it has been tentatively suggested by Walpole (1988a) that the karyotype for C. lectularius is the same as that described above for C. columbarius.

Johnson (1939) found that although the ratio of the 3rd/4th antennal segments differed significantly between insects he assumed were C. lectularius ($\bar{x}$ = 1,33; SD = 0,072; n = 484) and C. columbarius ($\bar{x}$ = 1,25; SD = 0,067; n = 255), the variation of the latter (1,10 - 1,45) was included in the former (1,05 - 1,65), so identification of individual specimens on this basis was not possible. He then tested the ratio head-width/3rd antennal segment length (HW/3A), and achieved a better separation (C. lectularius range = 1,10 -

1,89; $\bar{x}$ = 1,45; SD = 0,079; n = 1723. C. columbarius range = 1,40 - 2,10; $\bar{x}$ = 1,78; SD = 0,096; n = 409), the means being significantly different. However, the picture was complicated by bug populations found in animal houses which had intermediate HW/3A ratios (range = 1,30 - 1,74; $\bar{x}$ = 1,52; SD = 0,08; n = 347), the mean of this group differing significantly from the two others. Johnson (1939) stated that the bugs from animal houses had the "general facies" of C. lectularius, but he also did not exclude the possibility that bugs from an animal house in Kokstad, South Africa, were hybrids between the two species being considered. This speculation seems unjustified since the mean of the four insects from Kokstad ($\bar{x}$ = 1,64; SD = 0,053) falls within the range of C. lectularius. Also, no C. columbarius populations were reported in the African collections of Johnson (1939), or later by Usinger (1966), nor is it known whether hybrids would be intermediate as regards the HW/3A ratio. Johnson cross-bred the two species but did not present any morphological data on the hybrid progeny.

Johnson's (1939) field data thus showed a correlation between the HW/3A ratio of C. lectularius and human or non-human hosts. Laboratory work by the same author showed that C. columbarius did not acquire a HW/3A ratio characteristic of C. lectularius after three or four generations feeding on a rabbit, thus proving the irrelevance of avian or mammalian blood to this taxonomic character. Usinger (1966) found C. columbarius and

C. lectularius had HW/3A ratios of 1,71 and 1,45 after 30 generations being fed on rabbit. The two species respectively had HW/3A ratios of 1,72 and 1,47 after 30 generations of feeding on chicken and pigeon. The intermediate values of bugs found in animal houses as recorded by Johnson (1939) have not, therefore, been satisfactorily explained.

2.2.2 The examination of collections of Lectularius group made in KwaZulu

Materials and Methods

Ten male and ten female bugs of the Lectularius group were examined from each of eight huts in KwaZulu (Table 2). The insects were taken randomly from collections preserved in 70% alcohol and measured using an ocular micrometer in a dissecting microscope, when one unit of measurement = 0,033 mm.

Results

Table 2 shows the mean HW/3A ratios of male and female Lectularius group bugs from eight huts in KwaZulu. The average mean HW/3A ratio of male bugs differed significantly from the female ratio ($t = 5,03$; $P < 0,001$; d.f. = 158). The combined mean for the whole collection was 1,46 with a standard error of 0,01. The largest and smallest HW/3A ratios were recorded for a male at 1,24 and a female at 1,72 (Fig. 1, page 28).

Table 2. Mean HW/3A and standard errors of male and female Lectularius group bugs from eight huts in KwaZulu, ten bugs of each sex examined from each hut.

		Male bugs		Female bugs	
Hut	Location	Mean HW/3A	Standard error of mean	Mean HW/3A	Standard error of mean
1	27°13′S; 32°14′E	1,41	0,02	1,38	0,01
2	27°23′S; 32°23′E	1,42	0,02	1,45	0,02
3	27°23′S; 32°23′E	1,42	0,01	1,46	0,02
4	27°34′S; 32°10′E	1,42	0,02	1,49	0,01
5	28°05′S; 32°22′E	1,47	0,02	1,58	0,03
6	28°11′S; 32°18′E	1,46	0,02	1,50	0,02
7	28°13′S; 32°14′E	1,44	0,03	1,56	0,03
8	28°16′S; 28°E	1,41	0,02	1,48	0,03
A V E R A G E		1,43	0,01	1,49	0,01

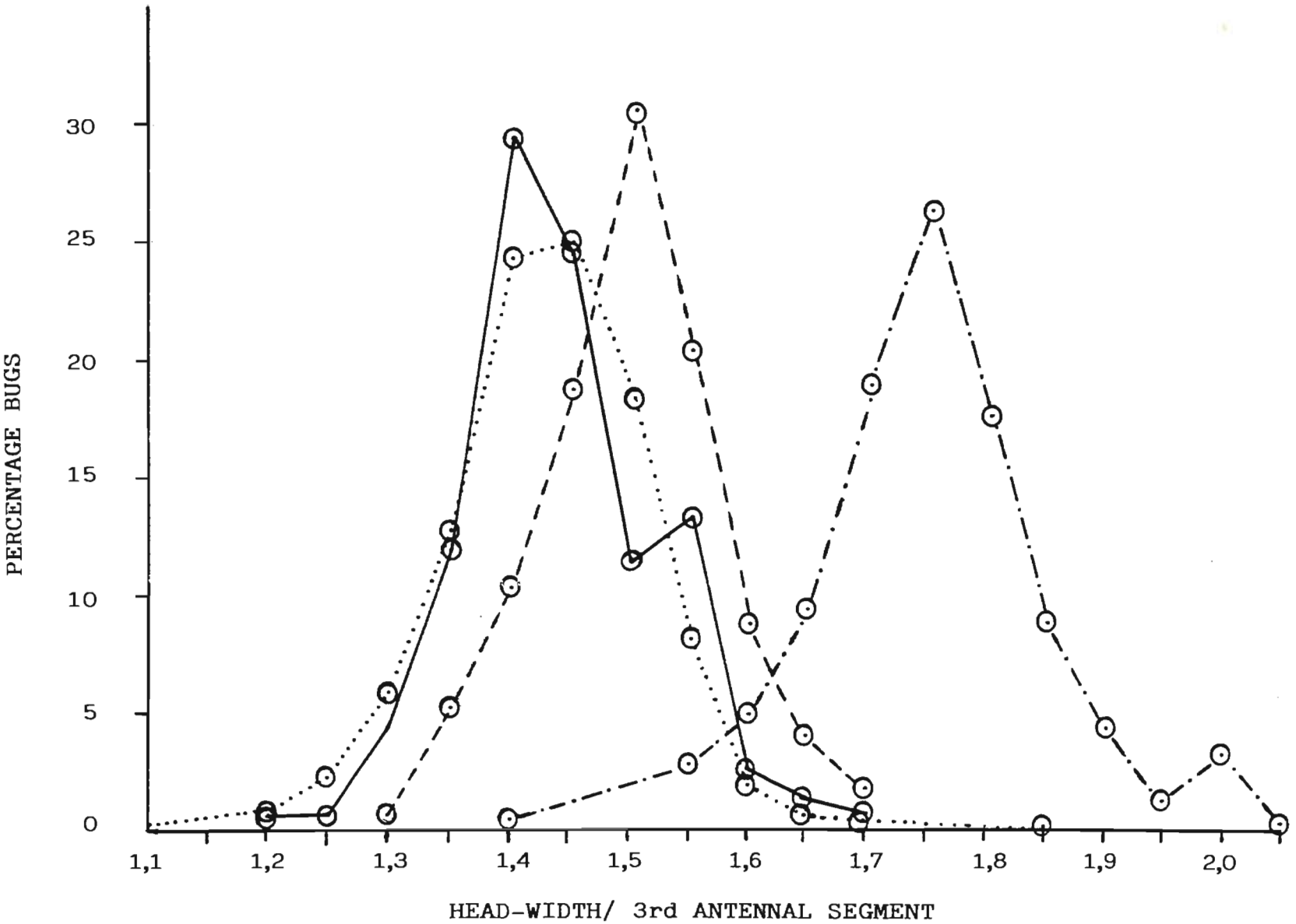
Fig. 1. Percentage distribution of HW/3A ratios in C. lectularius from KwaZulu (present work) and C. lectularius, bugs from animal houses and C. columbarius reported in Johnson (1939).

KwaZulu Lectularius group _____

Johnson (1939) C. lectularius .....

Johnson (1939) animal house bugs ----------

Johnson (1939) C. columbarius -.-.-.-.-.-.-.-.-

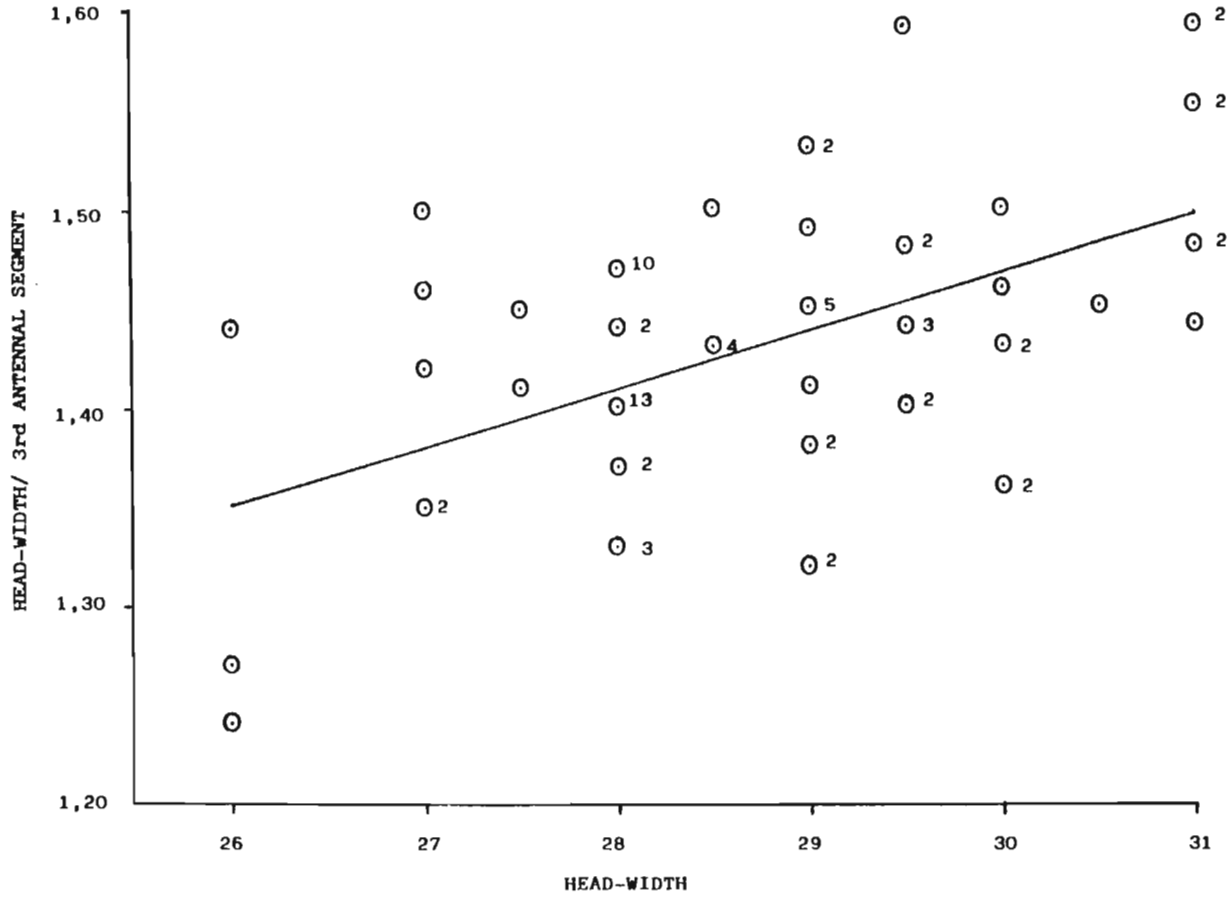

PERCENTAGE BUGS
30
25
20
15
10
5
0
1,1
1,2
1,3
1,4
1,5
1,6
1,7
1,8
1,9
2,0
HEAD-WIDTH/ 3rd ANTENNAL SEGMENT

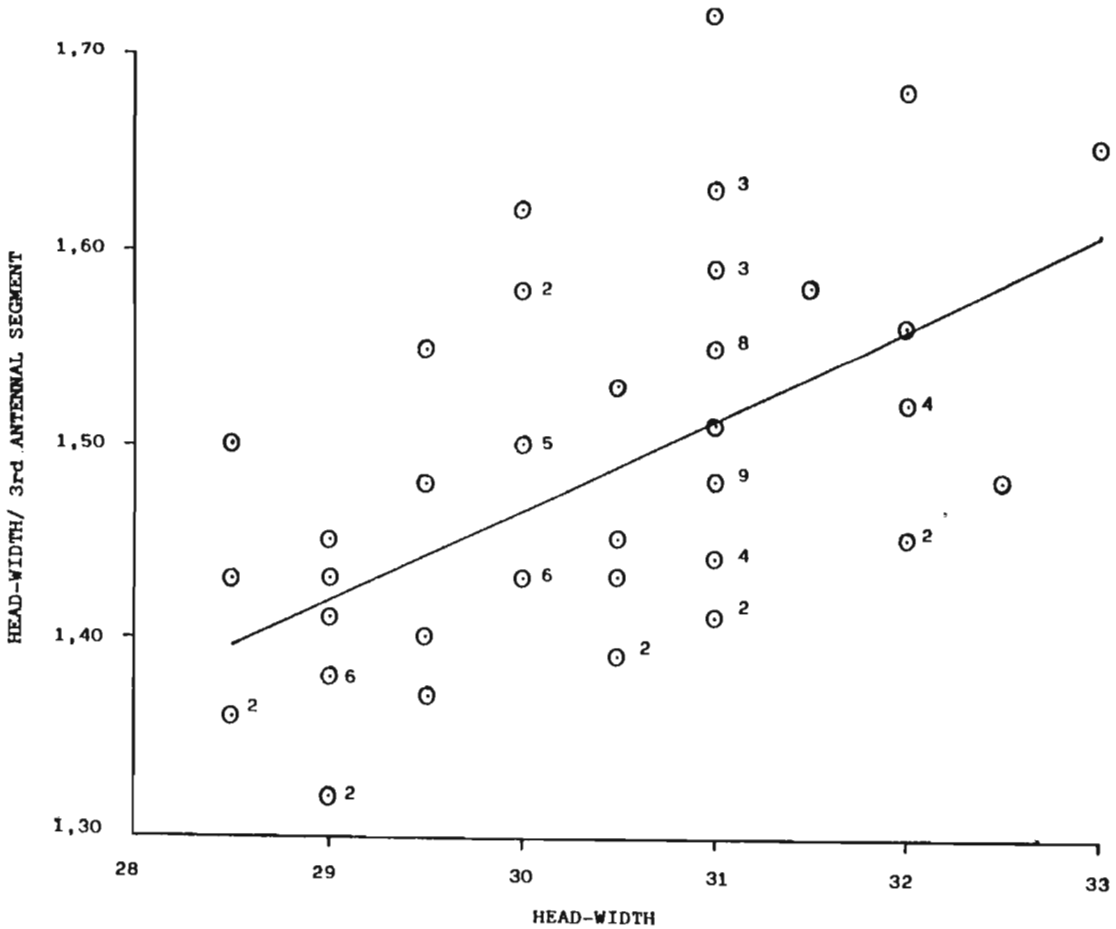
The HW/3A ratio increased with head-width in both male ($b = 0,03$; $y = 0,65 + 0,03x$; $t = 5,01$; $P < 0,001$; d.f. = 78) and female ($b = 0,05$; $y = 0,06 + 0,05x$; $t = 5,10$; $P < 0,001$; d.f = 78) bugs, the regression coefficients of the two groups not differing significantly from each other ($t = 1,78$; N.S.; d.f. = 156) (Fig. 2). The mean head-width of male bugs (28,64 units, s.e. mean = 0,132) was significantly less than that of females (30,48 units, s.e. mean = 0,115) ($t = 8,08$; $P < 0,001$; d.f. = 158).

Discussion

With the significantly larger HW/3A ratio of female bugs, the mean ratio for a population will be affected by sampling error favouring one of the sexes. Natural bug infestations also often have an excess of males. Overal & Wingate (1976) found that 63% (n = 460) of adult Stricticimex antennatus collected from a bat cave were male (chi-squared = 15,13; $P < 0,001$; d.f. = 1), as were 57% (n = 460) of C. lectularius caught in demon traps in an animal house by Mellanby (1939) (chi-squared = 4,45; $P < 0,05$; d.f. = 1). One of the six huts sampled by Newberry and Jansen (1986) had excess females (Hut 2: male % = 41; n = 288; chi-squared = 4,69; $P < 0,05$; d.f. = 1) and another had excess males (Hut 6: male % = 60; n = 590; chi-squared = 11,40; $P < 0,001$; d.f. = 1). Johnson's (1939) assertion that in his samples one sex did not usually predominate enough to significantly affect the mean HW/3A ratio is therefore unlikely to be generally true, and his results are unnecessarily confounded by a variable he could easily have removed.

Fig. 2. HW/3A ratio plotted against head-width for male (top) (y = 0,65 + 0,03x) and female (bottom) (y = 0,06 + 0,05x) C. lectularius collected in KwaZulu.


1,60
1,50
1,40
1,30
1,20
HEAD-WIDTH/ 3rd ANTENNAL SEGMENT
26
27
28
29
30
31
HEAD-WIDTH


1,70
1,60
1,50
1,40
1,30
HEAD-WIDTH/ 3rd ANTENNAL SEGMENT
28
29
30
31
32
33
HEAD-WIDTH

Female bugs are larger than males on average, and there is a tendency for the HW/3A ratio to increase with head-width. This means that large female C. lectularius are the most likely members of that species to score highly on the HW/3A ratio and thus possibly be confused with C. columbarius.

The mean HW/3A ratio for Lectularius group bugs from Kwazulu does not differ significantly from the mean for C. lectularius found by Johnson (1939) ($t = 1,20$; N.S.; $d.f. = 644$), but is significantly different from the ratio of the bugs found in animal houses reported by Johnson ($t = 7,85$; $P < 0,001$; $d.f. = 505$) and the insects Johnson designated as C. columbarius ($t = 36,19$; $P < 0,001$; $d.f. = 567$)(Fig. 1, page 28). Further evidence that the Lectularius group species in KwaZulu human habitations is C. lectularius comes from host association. Wild birds have never been noticed nesting in Zulu huts by the writer, no partition existing between the loft and the main interior of a hut. Chickens sometimes enter huts during the day, but only the occasional brooding hen has been observed to remain indoors at night, except in the case of delapidated huts with broken walls. Avian hosts are thus very seldom available, and infestations of C. columbarius are extremely unlikely. However, the human occupants of huts most definitely are bitten by bugs, the author also having been bitten during the day when entering huts where nobody has spent the night for over a week.

Morphological and behavioural evidence therefore indicates that the species of bug infesting human habitations in KwaZulu are C. hemipterus and C. lectularius. It is not known whether C. columbarius lives in association with birds in the same area, though it has not been recorded to date. For all the above reasons, it is assumed that all Lectularius group bugs found in Zulu huts in this study were C. lectularius.

2.3 THE SEPARATION OF NYMPHAL STAGES WITHIN SPECIES

2.3.1 The nymphal stages of C. lectularius

Usinger (1966) presented a key for distinguishing the five nymphal stages of C. lectularius, which is reproduced below.

1. One row of spines on each abdominal tergite.....First instar
   Two or more rows of spines on each abdominal tergite....2
2. Two rows of spines on each abdominal tergite.....Second instar
   Three or more rows of spines on each abdominal tergite, more-or-less distinct.....3
3. Last 2 antennal segments subequal.....Third instar
   Fourth segment distinctly shorter than third.....4
4. Hind margin of mesonotum not or scarcely concave at middle.....Fourth instar
   Hind margin of mesonotum distinctly, broadly concave at middle..... Fifth instar

A more detailed description of each nymphal stage in Usinger (1966) gave the average head-widths of each instar but without the range of measurements. It was therefore not known if head-width measurements could be used to reliably separate individual nymphs. Identification of nymphal stages by head-width is especially convenient for partial or damaged specimens where diagnostic bristles or antennae may be absent. A preliminary inspection of nymphal morphology suggested that the number of bristles along the lateral edges of the pronotum may be indicative of nymphal stage, so this character was also measured.

Materials and Methods

Nymphs were collected from a colony derived from C. lectularius caught from Zulu huts. The specimens were preserved in 70% alcohol then examined dry on a petri dish under a dissecting microscope at 30x magnification. The stage of each nymph was determined using Usinger's (1966) key, and the head-widths of ten of each instar were measured using an ocular micrometer accurate to 0,5 units (1 unit = 0,033 mm). The number of bristles visible on the lateral edges of the pronotum were counted on first to fourth stage nymphs, and the third and fourth antennal segments of third and fourth stage nymphs were also measured. Thirty nymphs of each instar were collected from Zulu huts infested with C. lectularius and head-widths were measured as above. The head-widths of thirty adults of each sex were also measured for purposes of the comparisons made in the Discussion.

Results

Tables 3 and 4 respectively record the head-widths of laboratory-reared and wild-caught nymphs of C. lectularius, the latter table also including adults. Table 5 presents measurements of numbers of lateral pronotal bristles, and 3rd/4th antennal ratios for third and fourth stage nymphs and Table 6 compares mean nymphal head-widths with those recorded by Johnson (1939) and Usinger (1966). The relative sizes of nymphs can be seen in Plate 1(page 2), and photographs in which lateral pronotal bristles can be counted appear in Plates 5, 6 and 7 (pages 46 to 48).

Discussion

Head-widths form a continuous series from first to fifth nymphal stages, with the range of each instar abutting or slightly overlapping the next. This measurement can therefore be useful for damaged specimens and for the majority of individuals which are in the middle of the range, but is not definitive. The comparison of mean nymphal head-widths recorded by Johnson (1939), Usinger (1966) and in this study (Table 6) indicates that mean nymphal head-width measurements were similar for C. lectularius from different areas. However, the 16% difference in mean head width between Johnson's (1939) largest and smallest groups of adult bedbugs is no doubt reflected in nymphal sizes, and local populations should be measured before individual head-width measurements are meaningful. The number of lateral pronotal bristles is a clear diagnositc character

Table 3. Head-widths (mm) of C. lectularius nymphs from a laboratory colony derived from insects captured from Zulu huts.

Stage (n = 10)	Range	Mean	Standard deviation
1st instar	0,36 - 0,40	0,37	0,014
2nd instar	0,45 - 0,48	0,46	0,014
3rd instar	0,50 - 0,61	0,55	0,030
4th instar	0,59 - 0,73	0,65	0,037
5th instar	0,73 - 0,83	0,77	0,030

Table 4. Head-widths (mm) of C. lectularius nymphs and adults caught in Zulu huts.

Stage (n = 30)	Range	Mean	Standard deviation
1st instar	0,31 - 0,40	0,35	0,019
2nd instar	0,40 - 0,48	0,43	0,024
3rd instar	0,48 - 0,59	0,54	0,025
4th instar	0,59 - 0,73	0,65	0,031
5th instar	0,78 - 0,86	0,82	0,021
Male	0,81 - 0,96	0,90	0,029
Female	0,89 - 0,99	0,94	0,028

Table 5. Bristles visible on the lateral edges of the pronotum of laboratory colony C. lectularius first to fourth stage nymphs, and ratio of lengths of 3rd/4th antennal segments in third and fourth stage nymphs.

Stage (n = 10)	Pronotal bristles Range	Mean	3rd/4th antennal segments Range	Mean
1st instar	8	8	-	-
2nd instar	15 - 18	17,0	-	-
3rd instar	24 - 31	26,4	1,0	1,0
4th instar	32 - 44	34,6	1,1 - 1,2	1,1

Table 6. Mean head-widths (mm) of nymphal stages of C. lectularius recorded in Usinger (1966), Johnson (1939) and from the present work.

Stage	Mean head-widths Johnson(1939)	Usinger(1966)	Present work Laboratory	wild-caught
1st instar	0,37	0,35	0,37	0,35
2nd instar	0,44	0,47	0,46	0,43
3rd instar	0,55	0,59	0,55	0,54
4th instar	0,66	0,68	0,65	0,65
5th instar	0,79	0,83	0,77	0,82

between first, second and third nymphal stages, but the ranges of the third and fourth nymphal stages abut.

From the above it can be said that Usinger's (1966) key may be supplemented by consideration of the number of lateral pronotal bristles, and head-width is a good general guide to nymphal stage.

The concave hind margin of the mesonotum of the fifth instar is very distinctive (Usinger, 1966), and the large head-width of this final nymphal stage is also indicative, but the lateral pronotal hairs are too numerous to count to be useful as a swift method of identification. The head-width range of South African fifth stage nymphs of C. lectularius (0,73 - 0,86 mm; Tables 3 and 4) overlap with the local adult range (0,81 - 0,99). To the naked eye, fifth stage nymphs have a more "thin-skinned" appearance and are generally of smaller size than adults, and can be distinguished with certainty from the adult stage by the absence of hemelytral pads.

2.3.2 The nymphal stages of C. hemipterus

Usinger (1966) and earlier works on C. hemipterus (Mellanby, 1935; Hase, 1930 & 1931) did not include information for the separation of the nymphal stages of this species. Work was therefore carried out to this end.

Materials and Methods

Eggs laid by wild-caught C. hemipterus females hatched in the laboratory and ten unfed first stage nymphs were preserved in alcohol. Attempts to feed the remaining nymphs on sedated, shaved guinea pigs failed, so they were fed on the author's arm. The nymphs were then isolated in individual tubes until moulting, when ten second stage nymphs were preserved and the remainder refed and re-isolated. This process was repeated until ten of each nymphal stage had been collected, though only three individuals remained to be reared to the fifth nymphal stage. The dry specimens were examined as above for the characteristics mentioned in Usinger's (1966) key for C. lectularius and for head-width and number of lateral pronotal hairs. Nymphs collected from huts infested by C. hemipterus were scored on the basis of characters found to be related to nymphal stage in the laboratory-reared insects, and the head-widths of 30 of each stage were measured. The head-widths of 30 adults of each sex were also measured for purpose of comparison (see Discussion).

Results

Tables 7 and 8 record the head-widths of laboratory-bred C. hemipterus nymphs, and wild-caught nymphs, the latter table also including adults. Table 9 considers lateral pronotal bristles, and the 3rd/4th antennal segment length ratio for third and fourth stage nymphs. The relative sizes of nymphs can be seen in Plate 2 (page 3), and photographs in which

Table 7. Head-widths (mm) of C. hemipterus nymphs reared in the laboratory from eggs laid by females collected from Zulu huts.

Stage (n = 10)	Range	Mean	Standard deviation
1st instar	0,36 - 0,40	0,38	0,014
2nd instar	0,43 - 0,46	0,44	0,014
3rd instar	0,51 - 0,56	0,53	0,030
4th instar	0,63 - 0,69	0,66	0,037
5th instar (n=3)	0,74 - 0,79	0,77	0,030

Table 8. Head-widths (mm) of C. hemipterus nymphs and adults collected from Zulu huts.

Stage (n = 30)	Range	Mean	Standard deviation
1st instar	0,36 - 0,38	0,37	0,008
2nd instar	0,41 - 0,50	0,45	0,020
3rd instar	0,53 - 0,58	0,55	0,015
4th instar	0,66 - 0,73	0,68	0,019
5th instar	0,78 - 0,89	0,82	0,030
Male	0,86 - 0,96	0,90	0,025
Female	0,89 - 0,97	0,93	0,025

Table 9. Bristles visible on the lateral edges of the pronotum of laboratory-raised C. hemipterus, first to fourth stage nymphs, and ratio of lengths of 3rd/4th antennal segments in third and fourth stage nymphs.

	Pronotal bristles		3rd/4th antennal segments	
Stage (n = 10)	Range	Mean	Range	Mean
1st instar	8	8	-	-
2nd instar	16	16	-	-
3rd instar	22 - 29	25,6	0,9 - 1,0	1,0
4th instar	34 - 38	35,2	1,0 - 1,2	1,1

lateral pronotal bristles can be counted appear in Plates 5,6 and 7 (pages 46 to 48).

Discussion

Usinger's (1966) key for C. lectularius nymphs is applicable to nymphal stages one to three of C. hemipterus in that these stages respectively bear one, two and three rows of bristles on the abdominal tergites. However, the third antennal segment of C. hemipterus fourth stage nymphs is not always longer than the fourth antennal segment, so this character cannot be used to distinguish third and fourth stage nymphs. The number of lateral bristles on the pronotum differed between the third and fourth stage nymphs, however (Table 9), and head-width ranges did not overlap (Tables 7 and 8), though the possibility that exceptional individuals infrequently occur cannot be excluded.

A tentative key for the identification of the nymphal stages of C. hemipterus may therefore be drawn up, subject to alteration on the basis of future work on collections from KwaZulu or other areas.

1. One row of spines on each abdominal tergite; head-width less than 0,40 mm; eight lateral pronotal bristles.....First instar

Two or more rows of spines on each abdominal tergite; head-width greater than 0,40 mm; more than eight lateral pronotal bristles.....2

2. Two rows of spines on each abdominal tergite; head-width between 0,41 and 0,50 mm; sixteen lateral pronotal bristles .....Second instar
Three or more rows of spines on each abdominal tergite; head-width greater than 0,50 mm; more than 16 lateral pronotal bristles.....3

3. Head-width between 0,51 and 0,58 mm; 22 to 29 lateral pronotal bristles.....Third instar
Head-width greater than 0,58 mm; more than 29 lateral pronotal bristles.....4

4. Head-width between 0,63 and 0,73 mm; 34 to 38 lateral pronotal bristles.....Fourth instar
Head-width greater than 0,73 mm; more than 38 lateral pronotal bristles.....5

5. Head-width between 0,74 and 0,89 mm; hind margin of mesonotum distinctly, broadly concave at the middle; no hemilytral pads.....Fifth instar

As in C. lectularius, the range of head-widths of South African fifth stage nymphs (0,74 - 0,89 mm; Tables 7 and 8) overlaps with the local adult range (0,86 - 0,97 mm), but the immature stage is distinguishable by its lack of hemilytral pads.

2.4 SPECIFIC SEPARATION OF IMMATURE STAGES

The eggs of C. lectularius and C. hemipterus may be distinguished because the greatest width of the egg of the former species is 1,5 - 1,6 times that of the cap end, and 1,8 times in the latter species (Usinger, 1966). Also, C. hemipterus eggs bear a coarsely reticulate pattern whereas C. lectularius eggs are moderately reticulated, more distinctly so near the cap end (Plate 4, page 45). In the present work, morphological features were required to quickly distinguish the nymphal stages of the two species and research proceeded to this end.

Materials and Methods

The bedbug collections mentioned in section 2.3 above were examined under a 30x dissecting microscope for readily distinguishable differences between the nymphal stages of the two species.

Results

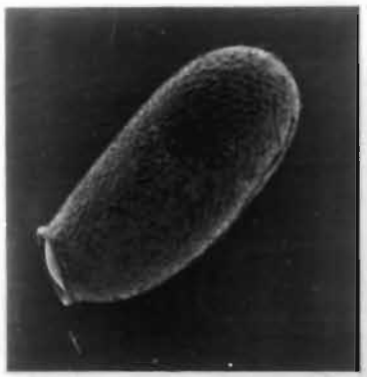
As described by Newberry (1988)(Chapter Six), the bristles along the lateral edges of the abdomen of C. hemipterus first stage nymphs alternate between long and shorter bristles a half or a third their length (Plate 5, page 46). Bristles in the same region of C. lectularius first stage nymphs alternately vary by about 20% in length and are more backwardly pointing than those of C. hemipterus. From stage two onwards, nymphs can be easily identified by the pronotal

bristles which are set almost at right angles to the pronotum in C. hemipterus but have a distinct backwards slant in C. lectularius. Though C. lectularius has a tendency to greater bodily size and width, and the distinct interspecific adult difference in the pronotum (Plate 3, page 8) becomes more apparent with advancing nymphal stage, same stage nymphs of the two species have a very similar appearance (Plates 5,6 and 7 pages 46 to 48).

Discussion

The minor interspecific differences between nymphs in size and shape are obscured by the considerable abdominal distention that occurs with feeding. The pronotum of C. lectularius is not consistently distinctive until the fourth nymphal stage and head-widths and lateral pronotal bristle numbers do not differ between same stage nymphs of the two species. However, the interspecific differences in nymphal chaetotaxy mentioned above enable rapid and accurate identification at 30x magnification.

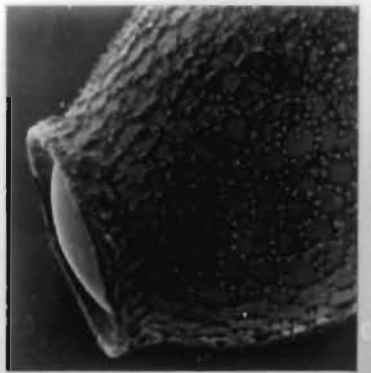
Plate 4. Fertile and sterile eggs of C. lectularius and C. hemipterus, without opercular caps, showing the heavier reticular patterning of the latter species especially in the sterile egg.



Cimex lectularius

fertile egg

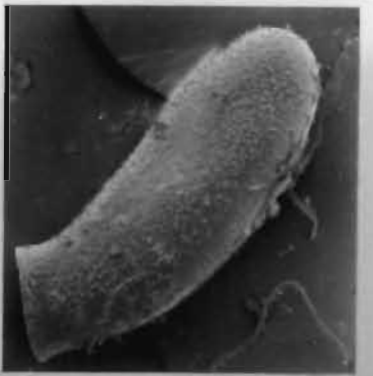
0,3 mm



Cimex lectularius

fertile egg
anterior

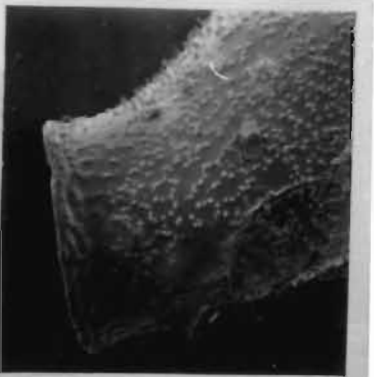
103 µm



Cimex lectularius

sterile egg

250 µm



Cimex lectularius

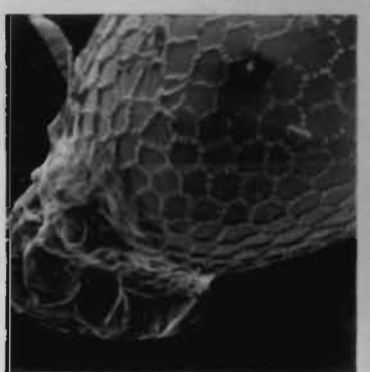
sterile egg
anterior

120 µm

Cimex hemipterus

fertile egg

0,3 mm



Cimex hemipterus

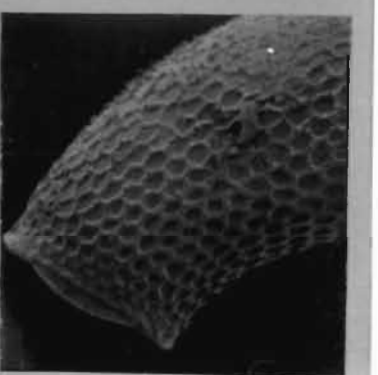
fertile egg
anterior

120 µm

Cimex hemipterus

sterile egg

250 µm



Cimex hemipterus

sterile egg
anterior

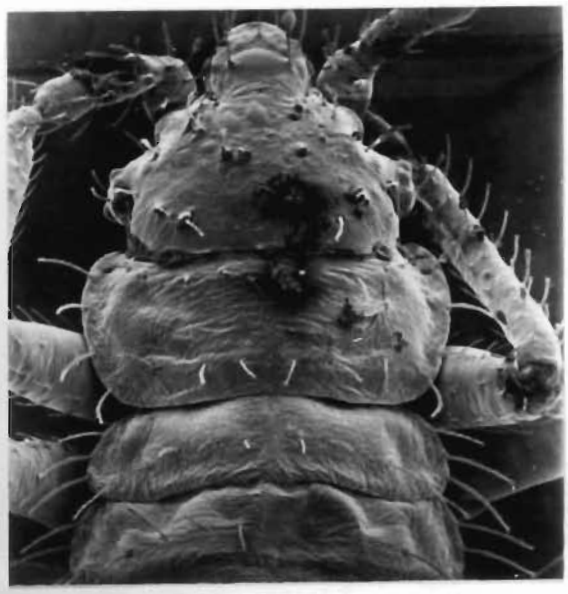
120 µm

Plate 5. First stage nymphs of C. lectularius and C. hemipterus the former species showing a more regular length of posterior lateral abdominal bristles, and both species having eight lateral pronotal bristles.

Cimex lectularius

first stage nymph

0,43 mm



Cimex lectularius

first stage nymph
anterior

200 µm

Cimex hemipterus

first stage nymph

0,38 mm

Cimex hemipterus

first stage nymph
anterior

200 µm

Plate 6. Second and third nymphal stages of C. lectularius and C. hemipterus showing the dorsal aspect, numbers of lateral pronotal bristles increasing with advancing life-stage, and the recurved bristles of the former species in comparison with the latter.

Cimex lectularius

second stage nymph

0,86 mm

Cimex lectularius

second stage nymph
anterior

0,30 mm

Cimex lectularius

third stage nymph

0,86 mm

Cimex lectularius

third stage nymph
anterior

0,30 mm

Cimex hemipterus

second stage nymph

0,75 mm

Cimex hemipterus

second stage nymph
anterior

0,30 mm

Cimex hemipterus

third stage nymph

1 mm

Cimex hemipterus

third stage nymph
anterior

0,30 mm

Plate 7. Fourth and fifth nymphal stages of C. lectularius and C. hemipterus showing the dorsal aspect, numbers of lateral pronotal bristles increasing with advancing life-stage, the recurved bristles of the former species in comparison with the latter and the distinct convexity in the posterior margin of the mesonotum of fifth stage nymphs of both species.

Cimex lectularius

fourth stage nymph

1 mm

Cimex lectularius

fourth stage nymph
anterior

0,30 mm

Cimex lectularius

fifth stage nymph

1,5 mm

Cimex lectularius

fifth stage nymph
anterior

0,75 mm

Cimex hemipterus

fourth stage nymph

1,5 mm

Cimex hemipterus

fourth stage nymph
anterior

0,5 mm

Cimex hemipterus

fifth stage nymph

1,5 mm

Cimex hemipterus

fifth stage nymph
anterior

0,5 mm

CHAPTER THREE

THE DISTRIBUTION OF THE BEDBUGS *Cimex lectularius* AND *Cimex hemipterus* IN NORTHERN NATAL AND KWAZULU

The domestic environments in which bedbugs thrive, namely human habitations and animal houses, are highly discontinuous. In rural KwaZulu, human dwellings are usually single-roomed huts three to ten metres apart. The intervening terrain is sandy or grassy soil which adds considerably to the effective distance or difficulty of the journey for a small crawling insect such as the bedbug. Many predators such as ants, centipedes and spiders abound in Africa, further limiting the chances that a bedbug would find and enter another hut by means of random search.

The seventy-one species of Cimicidae which live entirely in association with birds and bats also have very little prospect of finding a new host lair by their own active movement. In the Cimicidae generally there must therefore be strong selection for bugs not to wander from the nest, roost or house in which they dwell. This presupposes that Cimicidae possess an ability to detect the limits of their hosts' dormitory, and over distances of less than 75 cm this appears to be the case Marx (cited by Usinger, 1966).

Dispersal of cimicids from one discrete host environment to another is believed to occur principally by passive means. However, these insects are not adapted for secure attachment

to moving hosts and are rarely found on them. Passive dispersal is therefore uncommon among the zoophilic bugs. Ueshima (1968) found only two Paracimex bugs on 147 swiftlets caught in a cave in New Ireland. This author also found in New Britain that tunnels less than 50 m apart and used as nesting places for similar numbers of swiftlets could have very many, few, or no bugs at all. Usinger (1966) showed that Oeciacus does not migrate with its European host, the swallow, since it has never been found in nests in the tropics or in the southern hemisphere. In the case of the bedbugs, excellent opportunities for passive dispersal are afforded by transfer of furniture or luggage from one house to another. Mekuria (1967) detailed the many possibilities for the movement of bedbugs occasioned by everyday life in Ethiopia. Lewis (1949) could usually predict which species of bedbug would be found in a village if he knew the history of the tribe which lived there. The prevalence of bedbugs in the west country of Britain is believed to be a legacy of war time evacuations from the cities (Cornwell, 1974).

The arrival of bedbugs at a new location thus depends on the movement of their hosts or the hosts' belongings. The second aspect pertaining to distribution is whether the new environment is conducive to survival. Given the availability of a suitable host, bedbugs need an equable temperature (Omori, 1941) and the absence of serious threats such as efficient predators or effective host reaction, particularly

the use of insecticide (see Chapters Four, Five and Eight).

In the present work, two surveys were undertaken to discover the distribution of the bedbug species in the study area. The results are presented and discussed in Newberry et al. (1987) and Newberry & Mchunu (1989) on pages 53 to 58 of this thesis, and should be read at this point. It is clear from these surveys that C. hemipterus is able to thrive outside the tropics and is becoming more abundant there (see also Rosen et al., 1987). The subject of whether C. hemipterus displaces C. lectularius in huts infested by both species is considered in detail in Chapter Six.

Although some authors (Omori, 1941; Mekuria, 1967; Lewis, 1949) have stressed that C. lectularius and C. hemipterus within the same country are rarely found in the same locality, their conclusions are based on inadequate data. Mekuria (1967) stated that, "The number of samples and number of bedbugs collected, however, were not large enough to rule out the possibilities of the two species occurring together in any locality" in Ethiopia. Lewis (1949) collected "A few (specimens) at each village" in the Sudan, and received bugs from other collectors in order to compile his distribution map. The sort of surveys conducted by Mekuria (1967) and Lewis (1949) are unlikely to detect reliably whether both species exist in the same area, let alone the same building, though the latter situation was not unknown to Lewis (1949). Newberry et al. (1987) found by

intensive sampling that there were localities in KwaZulu infested by both bedbug species, and 10% of infested huts harboured both types of insect. Obviously the environmental requirements of the two species overlap, and intensive sampling in an area is necessary before it can be concluded that only one species is present.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1987) 81, 431-433

The occurrence of the bedbugs *Cimex hemipterus* and *Cimex lectularius* in northern Natal and KwaZulu, South Africa

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Abstract

Two species of anthropophilic bedbugs, *Cimex lectularius* and *C. hemipterus*, infest dwellings in KwaZulu, the known range of the latter extending to 28°15'S. *C. hemipterus* is the more common species in KwaZulu north of 27°15'. The pattern of distribution of *C. hemipterus* in South Africa is consistent with the species requiring a tropical climate, being a more recent invader than *C. lectularius*, and being able to thrive sympatrically with the latter species.

Introduction

MELLANBY (1935) showed that South Africa is part of the range of *Cimex lectularius*, the common bedbug, but not of *C. hemipterus*, the tropical bedbug. USINGER (1966) similarly recorded *C. lectularius* as widespread in South Africa, but also showed *C. hemipterus* on his map somewhere in the Natal/KwaZulu area, the details of the location not being given. Recent collections in the northern Transvaal (JUPP *et al.*, 1978, 1983) found only *C. lectularius*. The present work was undertaken to determine the prevalence of *C. hemipterus* in northern Natal and KwaZulu, where bedbugs present a problem to malaria control programmes (NEWBERRY *et al.*, 1984) and a potential health hazard concerning infective hepatitis caused by hepatitis B virus (JUPP *et al.*, 1983; OGSTON & LONDON, 1980).

Materials and Methods

All but 2 of the areas sampled lay between the Moçambique border with South Africa (26°45'S) and the Nkundusi/Mfekayi regions (28°15'S) east of 32°E (Fig. 1). The remaining 2 collections were made along the Nkwaleni valley (28°45'S, 31°30' to 40'E) and near Lake Cubhu (28°50'S, 31°57'E). With the exception of the sample from near Lake Cubhu, all bedbugs were found in areas where buildings are currently sprayed annually with DDT as part of an anti-malaria programme, and where DDT or BHC have been used for up to 40 years (DE MEILLON *et al.*, 1977; SMITH *et al.*, 1977).

Collections of bedbugs from farm labourers' quarters in the Nkwaleni valley, Natal, were from brick-walled buildings. All other collections were made in KwaZulu and, with very few exceptions, were from mud-walled huts with thatched or corrugated iron roofs. Bedbugs were found in dwellings by visual search of likely harbourages in beds, other furniture and cracks in walls, or by insecticidal knockdown. For the latter procedure, white spray sheets were spread on floors to catch bugs killed by various insecticidal treatments. Bedbugs in the third or later nymphal stage and adults were identified to species using morphological criteria (USINGER, 1966), especially the width and shape of the pronotum and the degree of development of the eyes. 11 huts which on visual search were found to contain both species of bug were resampled some months later.

Results

Our collections revealed a sympatric distribution of *C. lectularius* and *C. hemipterus* as far south as Nkundusi/Mfekayi at 28°15'S. Within this sympatric distribution, *C. lectularius* and *C. hemipterus* separately infested 196 (60·5%) and 96 (29·6%) huts respectively, with another 32 (9·9%) huts containing both species. From the northern limit of the study area, moving south at 15' intervals on the map (Fig. 1), *C. hemipterus* was found respectively in 94·3%; 70·6%; 39·4%; 8·9%; 8·3%; and 32·2% of infested huts. No. *C. hemipterus* were found in the Nkwaleni valley or near Lake Cubhu.

Spatial variation in relative abundance of bedbug species was also found on a smaller scale in Nkundusi/Mfekayi where an area about 20 km long was studied intensively (Fig. 2). *C. hemipterus* was found in 38·1% of infested huts along the main road (section D) and in 68·6% of infested huts in a busy area including the

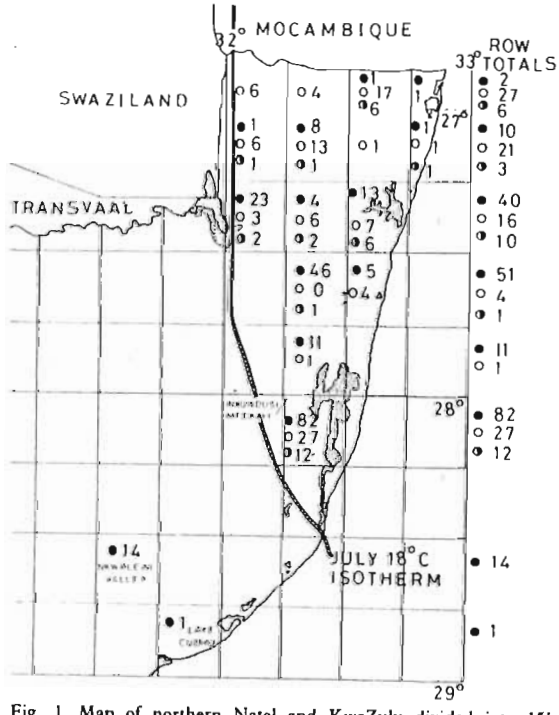

Fig. 1. Map of northern Natal and KwaZulu divided into 15' squares. Numbers adjacent to circles denote numbers of huts in each square that were infested with *C. lectularius* (black circles), *C. hemipterus* (white circles), and both species (black and white circles). Position of July 18°C isotherm from POYNTON (1964).

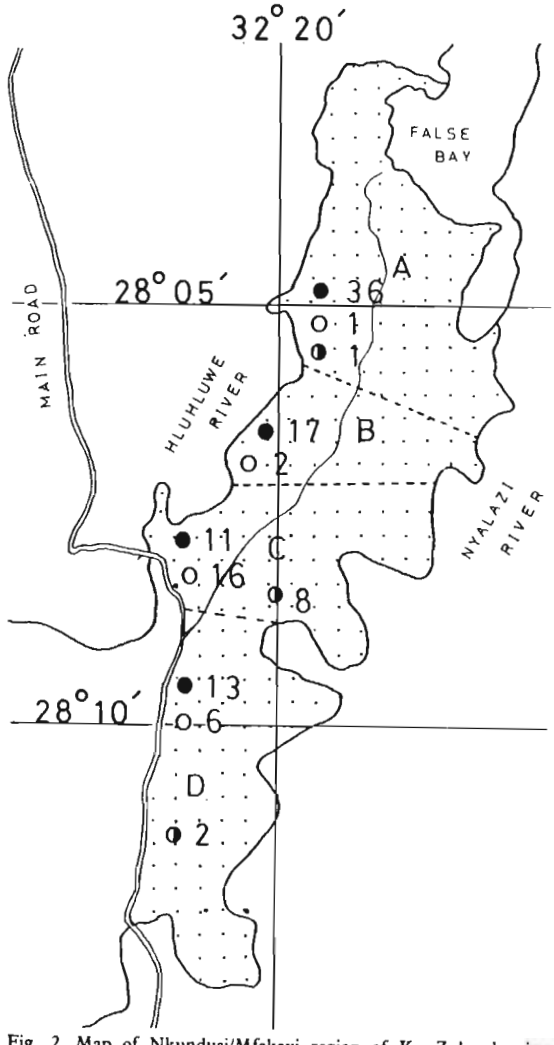

Fig. 2. Map of Nkundusi/Mfekayi region of KwaZulu showing numbers of infested huts in each of four areas. Black circles represent *C. lectularius*, white circles denote *C. hemipterus*, and mixed infestations are indicated by black and white circles.

local clinic and secondary school (section C). Further along the minor dirt road terminating at False Bay, *C. lectularius* was by far the most common species, *C. hemipterus* being found in only 10·5% and 5·2% of infested huts in sections B and A respectively.

In 3 huts (B to D, Table 1) where infestations with both species of bedbug had been found by visual search, collections at a later date revealed only *C. hemipterus* in each hut. In another hut (A) *C. hemipterus* were relatively much more plentiful in the second sample. 6 of the 7 other huts sampled twice (E to K) gave low numbers of *C. hemipterus* in one or both collections with (except for hut I) appreciable numbers of *C. lectularius* in both samples.

Discussion

C. lectularius and *C. hemipterus* were sympatric in the present study area north of 28°15′S (Fig. 1), and samples from 10% of infestations in this area contained both species. As samples were by no means exhaustive, the actual percentage of infested huts will be higher. These results contrast with those of MEKURIA (1967), who never found the two species in the same locality in Ethiopia, and of LEWIS (1949), who "seldom found" both species in one house in the Sudan.

Table 1—Collections of bedbugs during 1984 from huts which on a first visual search yielded both *C. lectularius* and *C. hemipterus*

Hut	Month	Numbers of *C. lectularius*	Numbers of *C. hemipterus*
A	Feb.	39	2
	Nov.	28	38
B	Apr.	18	36
	Jun.	0	23
	Nov.	0	37
C	Apr.	32	19
	Jun.	0	37
	Nov.	0	16
D	Feb.	51	1
	Dec.	0	64
E	Jul.	10	4
	Dec.	32	0
F	Jul.	19	4
	Dec.	45	4
G	Mar.	56	1
	Nov.	51	1
H	Apr.	51	2
	Jun.	49	0
I	Jul.	1	1
	Dec.	1	0
J	Jul.	1	23
	Dec.	0	13
K	Apr.	35	0
	Jun.	35	1

The question arises whether *C. hemipterus* is restricted to northern Natal/KwaZulu by climatic factors, or whether it is in the process of invading South Africa from Moçambique. Although *C. hemipterus* has now been found well south of the tropics in the present study, its known distribution in South Africa lies within the July 18°C isotherm (Fig. 1). As POYNTON (1964) remarked, the July 18°C isotherm is an indicator of a variety of climatic conditions which together constitute a tropical climate. MELLANBY (1935) did not demonstrate any aspects of the physiology of *C. hemipterus* that would preclude it from living in temperate countries. However, until the tropical bedbug is found thriving well outside the limits of tropical climate, the possibility that climatic factors limit its distribution cannot be ruled out.

C. hemipterus is most abundant relative to *C. lectularius* in the part of the study area north of 27°30′ (Fig. 1), where mean daily temperatures are higher for part of the year (THORRINGTON-SMITH *et al.*, 1978). However, the relative scarcity of *C. hemipterus* further south would also be expected if *C. hemipterus* were in the process of expanding its range from Moçambique. Opportunities for passive dispersal were very important in determining the distribution of *C. lectularius* and *C. hemipterus* in the Sudan (LEWIS, 1949), and MELLANBY (1935) explained the world-wide distribution of these species largely in terms of dispersal opportunities. From the present study there is reason to believe that *C. hemipterus* has

not yet dispersed throughout the whole range in South Africa which is suitable for it. For example, in the Nkundusi/Mfekayi area (Fig. 2), *C. hemipterus* was common in huts along the main road and around a school and clinic, but rarely found away from the main flow of human traffic. By contrast, *C. lectularius* was ubiquitous in the area and commonly found in huts in less frequented places.

MELLANBY (1935) concluded that *C. lectularius* is better able to withstand starvation and desiccation than *C. hemipterus*, breeds faster at temperatures below 30°C, and should thrive at least as well as *C. hemipterus* in a tropical climate. It is therefore surprising that 3 huts initially containing appreciable numbers of *C. lectularius* as well as *C. hemipterus* yielded only the latter species some months later (Table 1). If replacement of *C. lectularius* by *C. hemipterus* did occur, a mechanism which could explain it was mentioned by USINGER (1966), who related that transfer of sperm during mating between male *C. hemipterus* and female *C. lectularius* had fatal consequences for the latter, and that this phenomenon has been suggested as the reason for the absence of *C. lectularius* from some areas of the tropics. Male bedbugs certainly can be very unselective in their mating behaviour in the laboratory (USINGER, 1966), but direct evidence from the field is needed to confirm that interspecific mating actually occurs in nature frequently enough to exterminate *C. lectularius* infestations.

The annual spraying of dwelling places with DDT may have facilitated the spread of bedbugs by the removal or reduction in numbers of their predators (NEWBERRY *et al.*, 1984). It is not known whether there are differences between the two species in levels of resistance to DDT which may affect distribution.

Acknowledgements

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Changes in the relative frequency of occurrence of infestations of two sympatric species of bedbug in northern Natal and KwaZulu, South Africa

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Abstract

Between 1983 and 1988 the frequency of *Cimex hemipterus* infestations relative to *C. lectularius* increased near the Mozambique border and around main roads in the Nkundusi-Mfekayi area of South Africa (28°15'S; 30°23' E). In areas where both species were common, the observed number of doubly-infested huts equalled the expected. A hut infested by both bedbug species showed a significant change to a higher proportion of *C. hemipterus* in the adult population over 2 months. The general increase in *C. hemipterus* is thought to be due to the large influx of migrants into South Africa in recent years.

Introduction

NEWBERRY *et al.* (1987) mapped the distribution of the bedbugs *Cimex lectularius* and *C. hemipterus* in northern Natal and KwaZulu, South Africa, and concluded that the latter species appeared to be in the process of invading the area. Considerable, largely unofficial, human migration from Mozambique has occurred over the past few years giving excellent opportunities for the passive dispersal of bedbugs into northern Natal and KwaZulu. *C. hemipterus* is believed to be common in Mozambique (USINGER, 1966), so the possibility existed that significant changes in the relative abundance of the 2 species could have occurred in the area.

Materials and Methods

The study area lay between the Mozambique border with northern Natal and KwaZulu (26°25' S) and 28°25' S, east of 32° E (Fig. 1). This is the region studied by NEWBERRY *et al.* (1987), excluding the sites of the 2 most southerly collections. Intensive sampling was again done in the Nkundusi-Mfekayi region (Fig. 2), which includes the area studied by NEWBERRY *et al.* (1987). Since this earlier work huts in parts of sections A and B (Fig. 2) had been successfully treated with an organophosphate insecticide by the KwaZulu Department of Health, and no collections were made in these sprayed areas. Collections from human habitations were all made by visual search of furniture, cracks in walls, and beds. Identifications were made usually on live adults and larger nymphs, using morphological criteria (USINGER, 1966).

Huts were recorded as infested with either, or both, species, or uninfested, to enable calculations of the expected frequency of doubly-infested huts in areas where both species were common.

One hut with an appreciable infestation of both bedbug species was monitored from February to April 1987, but was then treated with insecticide by the Health Department.

Results

Comparing Fig. 1 in this paper with Fig. 1 of NEWBERRY *et al.* (1987), there was a very significant increase in the relative abundance of *C. hemipterus* in the Mozambique border area (A–H in Fig. 1) ($\chi^2=35{\cdot}76$, $P<0{\cdot}001$). Further south (Fig. 1, I–K),

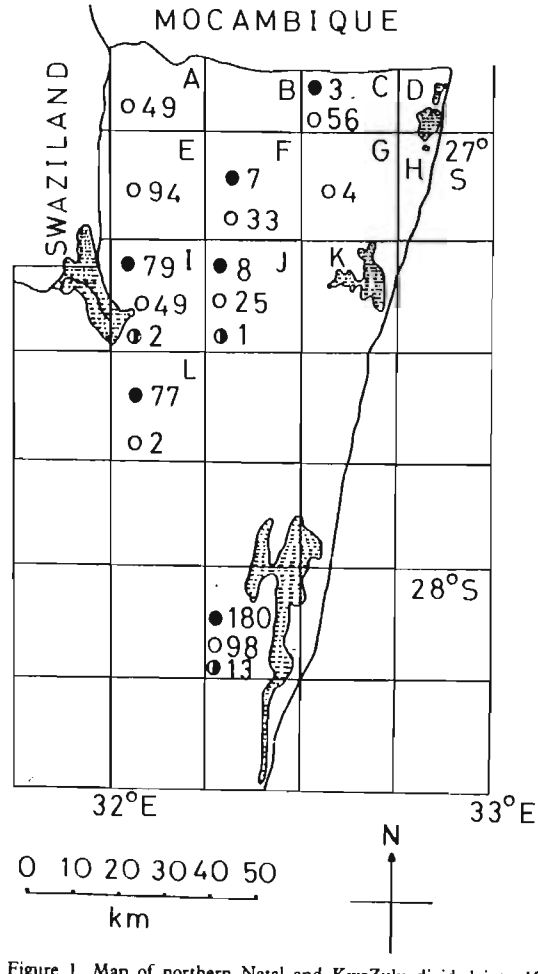

Figure 1. Map of northern Natal and KwaZulu divided into 15' squares, showing numbers of huts in each square infested with *Cimex lectularius* (black circles), *C. hemipterus* (white circles) and both species (split circles).

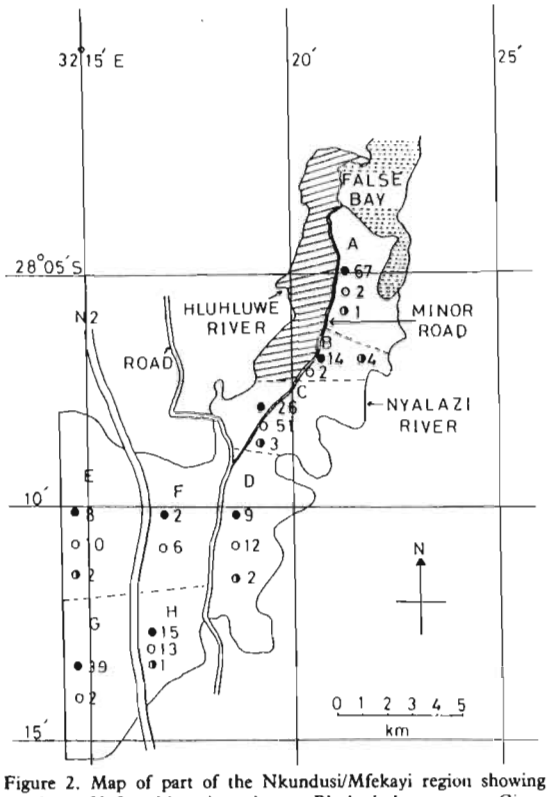

Figure 2. Map of part of the Nkundusi/Mfekayi region showing numbers of infested huts in each area. Black circles represent *Cimex lectularius*, white circles denote *C. hemipterus*, and mixed infestations are indicated by split circles. In the shaded section bedbugs had been eradicated by application of an organophosphate insecticide.

Table 1. Collections of bedbugs from a hut infested by *Cimex hemipterus* and *C. lectularius* during 1987

Dates of collection	No. of adults (%) *C. hemipterus*	*C. lectularius*
10–25 February	93 (75·6)	30 (24·4)
24 March	52 (92·9)	4 (7·1)
9 April	221 (94·0)	14 (6·0)

tions from square I (Fig. 1) were relatively richer in huts infested with *C. lectularius* than those from square J, both in 1984 and 1986 ($\chi^2=7{\cdot}51$, $P<0{\cdot}01$; $\chi^2=14{\cdot}15$, $P<0{\cdot}001$, respectively). However, there was a significant tendency for a relative increase in *C. hemipterus* in square I since the previous study ($\chi^2=5{\cdot}22$, $P<0{\cdot}05$), but not in square J ($\chi^2=1{\cdot}38$).

In the Nkundusi-Mfekayi region (Fig. 2), 4·5% of huts were infested with both species, whereas only 0·6% of huts contained double infestations in areas sampled to the north. These percentages are significantly lower than the 9·9% found in both areas in the previous study ($\chi^2=4{\cdot}40$, $P<0{\cdot}05$, and $\chi^2=38{\cdot}10$, $P<0{\cdot}001$, respectively). There was a significant increase in the relative abundance of *C. hemipterus* in areas, B C and D ($\chi^2=5{\cdot}07$, $P<0{\cdot}05$). However, area A remained dominated by *C. lectularius* ($\chi^2=0{\cdot}05$; not significant). *C. lectularius* infestations increased, compared to those due to *C. hemipterus*, as one proceeded along the minor road to False Bay (Fig. 2). This ratio was higher in area C than in area B ($\chi^2=12{\cdot}13$, $P<0{\cdot}001$), and higher in area B than in area A ($\chi^2=9{\cdot}03$, $P<0{\cdot}01$). Both trends were present in the earlier survey (NEWBERRY *et al.*, 1987), though only the B-C comparison was significant ($\chi^2=11{\cdot}10$, $P<0{\cdot}01$).

Collections for 20 km further south of the study area, along the N2 highway, revealed that 21 huts out of 129 checked were infested with *C. lectularius*, none with *C. hemipterus*. In areas A and G of the Nkundusi-Mfekayi study area (Fig. 2), *C. hemipterus* accounted for less than 5% of the total number of infestations. In each of the remaining areas (Fig. 2), both species were strongly represented with neither contributing less than 25% of the infestations discovered. Considering these areas, 86 of 695 huts (12·4%) were infested with *C. lectularius*, and 106 (15·3%) with *C. hemipterus*. Assuming random distribution of infestations, the expected number of doubly-infested huts in these areas is 13·12, which agrees very well with the observed number of 13.

One hut infested by both species showed a relative increase of adult *C. hemipterus* occurring over one month (Table 1). Comparing the February collection with those in March and April, the change was highly significant ($\chi^2=27{\cdot}95$, $P<0{\cdot}001$).

Table 2. Numbers of huts infested with *C. lectularius*, *C. hemipterus* or both species, in northern KwaZulu during 1986–1987

Area	No. of huts examined	No. of infested huts (%) *C. lectularius*	*C. hemipterus*	Both species	Total
A–H[a]	1364	10 (0·7)	236 (17·3)	0 (0)	246 (18·0)
I–L[a]	1120	164 (14·6)	76 (6·8)	3 (0·3)	243 (21·7)
Nkundusi/Mfekayi	1123	180 (16·0)	98 (8·7)	13 (1·2)	291 (25·9)

[a]See Fig. 1.

the tendency was also towards an increase in *C. hemipterus*, but it did not achieve statistical significance ($\chi^2=3{\cdot}03$). *C. lectularius* remained very predominant in collections made at the latitude of square L (Fig. 1). More localized differences in the relative abundance of the bedbugs were also found. Collec-

Infestation rates ranged between 18 and 26% of the 3607 huts examine in all the areas sampled (Table 2).

Discussion

Lewis (1949) could usually predict which species of bedbug would be found in a Sudanese village if he

knew the history of the movements of the tribe living there. The data presented in this paper and the previous one (NEWBERRY *et al.*, 1987) indicate that passive dispersal was the only likely cause of the relative increase in *C. hemipterus* infestations in the Mozambiquan border area and the busier areas of Nkundusi and Mfekayi. Although no details of population movements are available, it is reasonable to assume that areas with very few *C. hemipterus* infestations (L, Fig. 1; A and G, Fig. 2) simply have not had the insect introduced.

The incidence of samples containing both bedbug species from the same hut is lower than that found by NEWBERRY *et al.* (1987), probably due to differences in sampling techniques. No insecticidal 'knockdown' was used in the present work, so light infestation of a second species may have been missed in some huts. There is no reason to suspect that either species was favoured by this change in sampling method.

The infestation rate of 22% is also lower than the 44% found by NEWBERRY *et al.* (1984). However, the earlier study scored current and recent infestations on the presence of bugs, exuviae or egg shells, whereas the present work considered only current infestations as evidenced by live adult or nymphal bedbugs.

OMORI (1939) found that interspecific mating in the laboratory shortened the life span and disrupted the production of fertile eggs of female *C. lectularius*, but had no effect on female *C. hemipterus*. Should interspecific mating occur in the field, one would expect the numbers of *C. hemipterus* in a mixed infestation to increase relative to the numbers of *C. lectularius* as the reproductive potential of the latter species was eroded. This has been demonstrated by NEWBERRY *et al.* (1987) and the present paper. However, one would also expect the observed number of double infestations to be less than expected if double infestations had a limited life span of less than that of single species infestations. This was not so in the present work, though there may well be other relevant factors, such as frequent reintroductions of *C. lectularius* into doubly-infested huts. Also, the effect of a few *C. hemipterus* on a very large *C. lectularius* infestation is likely to be slight. More needs to be known about the frequency and effects of interspecific mating in the field before its effects on the distribution of *C. lectularius* can be assessed.

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CHAPTER FOUR

THE PRODUCTION OF EGGS BY *Cimex lectularius* AND *Cimex hemipterus*

4.1 INTRODUCTION

The rate at which an animal species is able to reproduce is important to its survival. The length of time between mating and laying eggs, the rate at which eggs are produced, their number and fertility, all influence the size of the bedbug population. If insufficient fertile eggs are produced per unit time to replace individuals lost to starvation, predation, host response to biting etc., the population will die out. The cosmopolitan distribution of *C. lectularius*, and restriction of the range of *C. hemipterus* to the tropics, or close to them (Usinger, 1966; Rosen *et al.*, 1987; Newberry *et al.*, 1987) suggests that *C. hemipterus* cannot survive in temperate regions. Laboratory observations (Chapter One) support the hypothesis that *C. hemipterus* is less fecund than *C. lectularius* at all but the highest temperatures tested. However, the two bedbug species have never been compared on any physiological character when living in the wild, so the possible influence of artificial laboratory conditions had not been assessed.

In this study, the process of egg production in both species was studied in the laboratory and in Zulu huts where bugs had existed under entirely natural conditions prior to

egg laying. A comparison of laboratory and field assessments of egg production was therefore possible which would reflect on the relevance of laboratory findings to the understanding of the abundance and distribution of bedbugs.

4.2 PREOVIPOSITION PERIOD AND DAILY RATE OF EGG PRODUCTION

Materials and Methods

Wild-caught fifth stage nymphs were isolated in tubes until adult emergence. The virgin insects were fed in single sex groups, then divided into ten mating pairs, each pair being placed in a 50 x 15 mm glass tube with a cardboard rectangle to rest on. Bugs were kept in an incubator set at 25 degrees C and about 30% relative humidity, and monitored over 24 days for egg production.

Results

Three C. hemipterus died early in the experiment and were discarded. Three more females of the same species died before the 24 day period elapsed, but after all bugs had stopped producing eggs. The results over the 14 days during which eggs were laid are given in Tables 10 and 11. Eggs were first laid by both species four days after mating and feeding, when two of the ten C. lectularius females and all seven C. hemipterus oviposited. Both species laid their last eggs on the fourteenth day of egg production. Cimex lectularius and C. hemipterus respectively laid 63 and 61%

of their total egg output by the fourth day of production, 92 - 93% by the eighth day, and averaged 13,00 and 12,14 eggs over the whole period. No difference existed between the species in the ratio of number of bugs to eggs produced (chi-squared = 0,02; d.f. = 1; N.S.).

Discussion

Interpolating Omori's (1941) findings, the preoviposition period of C. lectularius is 5,3 days at 25 degrees C, which contrasts with the figure of 3,0 days given by Johnson (1941), which is the same length of time found at a temperature three degrees higher by Davis (1964). The present finding of 4,8 days agrees more closely with Omori (1941) than Johnson (1941). The preoviposition period for C. hemipterus at 25 degrees C interpolates from Omori (1941) as 4,8 days, as opposed to the 4,0 days found in the present work. The differences between Johnson's and Omori's findings may be due to different strains of the bedbugs being used (Yanovski & Ogston, 1982), only approximate temperature control during one or both experiments, or both these variables.

The present findings confirm Omori's (1941) statement that the preoviposition period of C. hemipterus is shorter than that of C. lectularius. The subsequent rate of production of eggs shows that C. hemipterus was not slower, even at the moderate temperature of 25 degrees C, and therefore not at a disadvantage to C. lectularius in this respect. Both species

Table 10. Eggs laid at 25°C by ten once-mated C. lectularius females after one blood meal.

Bedbug no.	Days of egg-laying period														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
	No. of eggs														
L1	0	1	2	3	1	2	2	0	0	0	0	0	0	0	11
L2	0	2	3	4	2	2	1	0	0	0	0	0	0	0	14
L3	3	4	2	2	0	0	0	0	1	0	0	0	0	0	12
L4	2	4	3	1	0	0	0	0	1	1	0	0	0	0	12
L5	0	3	2	2	4	0	2	1	0	1	0	0	0	1	16
L6	0	1	3	2	4	2	1	1	0	0	1	0	1	0	16
L7	0	3	4	1	3	1	1	0	0	0	0	1	0	0	14
L8	0	2	2	2	1	0	0	0	0	0	0	0	0	0	7
L9	0	3	2	4	1	1	2	0	0	0	1	1	0	0	15
L10	0	2	4	3	2	1	1	0	0	0	0	0	0	0	13
TOTAL	5	25	27	24	18	9	10	2	2	2	2	2	1	1	130
Accumulated percentage	3,9	23,4	44,5	63,3	77,3	84,4	92,2	92,3	93,8	95,4	96,9	98,5	99,2	100	

Table 11. Eggs laid at 25°C by seven once-mated C. hemipterus females after one blood meal.

Bedbug no.	Days of egg-laying period														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
	No. of eggs														
H1	3	2	1	3	1	0	2	2	0	0	0	0	0	0	14
H2	4	4	2	2	1	0	0	2	2	0	0	0	0	0	17
H3	3	1	1	1	0	0	1	1	0	0	0	0	1	1	10
H4	3	2	1	2	2	2	2	0	0	0	0	0	0	0	14
H5	2	1	0	2	0	0	1	2	0	0	0	0	0	0	8
H6	3	2	2	1	1	1	1	0	1	0	0	0	0	0	12
H7	2	1	1	0	3	1	1	0	1	0	0	0	0	0	10
TOTAL	20	13	8	11	8	4	8	7	4	0	0	0	1	1	85
Accumulated percentage	23,5	38,8	48,2	61,2	70,6	75,3	84,7	92,9	97,6	97,6	97,6	97,6	98,8	100	

produced over 90% of their eggs at one day past the mid point of their egg laying time. Davis (1964) reported the same phenomenon for C. lectularius at 28 degrees C, which raises the possibility that this pattern of egg laying is constant over a range of temperatures. As the number of eggs per bug did not differ significantly between the species, and the rate of production was the same, C. hemipterus had a slight advantage over C. lectularius when laying eggs at 25 degrees C because of its shorter preoviposition period.

4.3 EGG PRODUCTION IN THE LABORATORY BY BEDBUGS COLLECTED FROM ZULU HUTS

Materials and methods

During November and December 1986, female bedbugs were collected from Zulu huts and placed individually into tubes containing pieces of crumpled paper for egg laying. Five huts containing C. lectularius were sampled, and three huts infested with C. hemipterus. The insects were brought to the laboratory and held in an insectary maintained at an average of 25 degrees C and ambient humidity. Fertile eggs were counted eleven days later.

Results

Of bugs which laid eggs and lived for 11 days or longer, 108 C. lectularius laid 1393 eggs, whilst 86 C. hemipterus laid 1138 eggs. The means for the two species were 12,90 and 13,23 per bug respectively, the difference in egg production

being non significant (chi-squared = 0,03; d.f. = 1; N.S.). When the 18 C. lectularius and 14 C. hemipterus which did not lay are also considered, the means become 11,06 and 11,38 per bug respectively, the egg production of the two species still being similar (chi-squared = 0,09; d.f. = 1; N.S.).

Discussion

Over 96% of the eggs of both species are laid by the 11th day, if no more feeding takes place (Tables 10 and 11). The above results therefore indicate the number of eggs each species can develop under natural conditions of mating and feeding during late spring and summer months. As in section 4.2, C. hemipterus was not found to be at a disadvantage.

4.4 NUMBERS OF EGGS PRODUCED BY CAPTURED BUGS HELD IN A MUD HUT

The summer study was carried out in February 1987, when female bedbugs in two huts infested by different species were collected and placed individually in tubes containing small pieces of crumpled paper. The insects were retained in their huts of origin for 11 days, then taken to the laboratory where the eggs of surviving bugs were scored. The daily maximum and minimum temperatures recorded at the nearby Makatini Research Station were noted for the period.

The spring study was carried out in October 1988. Bugs of both species collected from Zulu huts were sorted in the

laboratory into individual tubes and taken the next day to remain in a hut (itself infested with C. hemipterus) for eight days. After this, the bugs were put into fresh tubes and taken back to the laboratory where the insects were scored for mortality. The bedbugs were held for a further eight days, and then the eggs of live bugs and those which had been alive at the end of their stay in the hut, were scored. Maximum and minimum temperatures were recorded in the hut and laboratory during the days the animals were held in these places.

Results

See Tables 12 and 13.

Discussion

The two bedbug species living in their natural habitats but confined for a period of egg laying did not show a difference in egg production at average temperatures of 27,9 and 22,5 degrees C.

Temperatures recorded inside huts will not necessarily be those experienced by bedbugs which actively seek optimum conditions in their environment (Omori, 1941). Many bedbugs live in the blankets and mattresses of their hosts, and will therefore benefit from the bodily warmth of hut occupants during cold nights. Copulation, egg laying and hatching, and nymphal development of C. hemipterus may therefore be possible in winter during the warmest times of the day, and

Table 12. Female bedbugs collected in February 1987 from Zulu huts and held in the huts for 11 days. Temperatures calculated from recordings at the Makatini Research Station.

Mean max. temp.: 33,7 Mean min. temp.: 22,0 Average temp.: 27,9 degrees C

	C. lectularius	C. hemipterus
No. of bugs captured	18	22
No. of bugs laying eggs	16	16
No. of fertile eggs laid	215	255
Mean no. of eggs laid per fertile bug	13,44	15,94
Mean no. of eggs per bug	11,94	11,59

Table 13. Female bedbugs collected in October 1988 from Zulu huts and held overnight in the laboratory, eight nights in a Zulu hut, and eight nights in the laboratory. Temperatures taken on site. Figures for the first nine days in parentheses.

	Mean max. temp.	Mean min. temp.	Average
first 9 days	26,3 deg. C	18,7 deg. C	22,5 deg. C
Second 8 days	27,5 deg. C	21,4 deg. C	24,4 deg. C

	C. lectularius	C. hemipterus
No. of bugs captured	31	27
No. of bugs laying eggs	26	26
No. of fertile eggs laid	448 (437)	422 (401)
Mean no. of eggs laid per fertile bug	17,23 (16,80)	16,23(15,42)
Mean no. of eggs per bug	14,45 (14,10)	15,63(14,85)

close to the host at night. More work is needed to elucidate the population dynamics of the two bedbug species under natural conditions, especially during the winter months.

4.5 DISCUSSION OF SECTIONS 4.2 - 4.4

Omori (1941) found that female C. lectularius laid more eggs per day on average than did C. hemipterus at moderate constant temperatures (means 3,28 and 2,04 at 22 degrees C; 5,39 and 4,31 at 27 degrees C respectively). Walpole (1988) found C. lectularius and C. hemipterus laid averages of 8,20 and 2,56 eggs per week respectively at 28 degrees C. My own laboratory comparisons at 25 degrees C (Chapter Six, page 121) resulted in weekly yields of 5,90 and 2,27 eggs per week respectively.

In all the above results, C. lectularius produced more eggs per bug than did C. hemipterus. In contrast to these findings, the two species laid eggs in the same quantity and at the same rate at 25 degrees C after one blood meal. (Section 4.2). The ratio of female bugs to eggs laid also did not differ significantly between species when wild-caught bugs were allowed to lay without further feeding, either in the laboratory (Section 4.3) or in Zulu huts (Section 4.4). These observations give no indication that C. lectularius is superior to C. hemipterus in egg production even at temperatures as low as 22,5 degrees C (Table 13).

The question is therefore raised whether the relatively poor egg laying performance of C. hemipterus when maintained in the laboratory for a number of weeks is in fact an artifact of its poor adaptation to artificial conditions. In my experience, and that of Walpole (D E Walpole, pers. comm.*) it is far more difficult to establish and maintain colonies of C. hemipterus than of C. lectularius from wild-caught material. It may be that C. hemipterus is better adapted to sub-tropical climates than laboratory studies have so far indicated.

4.6 STERILE EGGS

Some eggs which appear normal at the time of laying soon shrivel, become opaque and discoloured, and do not develop an embryo. These eggs, called "taube" by Hase (cited by Omori, 1941), "imperfect" (Cragg, 1923), or "abnormal" (Omori, 1939) are normal eggs which have not been fertilized and lack a serosa (Mellanby, 1939). Such eggs are thus different from eggs which maintain a normal appearance but fail to hatch due to some post-zygotic malfunction.

Virgin female C. lectularius may lay up to two sterile eggs (Davis, 1964). Mated females of both species occasionally lay one or more sterile eggs amid batches of normal eggs, the percentage rising at extreme temperatures (Omori, 1941).

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Mated females which are not re-fertilized for some weeks lay an increasing number of sterile eggs as sperm from their last mating is depleted. Finally, only sterile eggs are produced and egg laying ceases entirely soon after this (Davis, 1964). Female bedbugs of both species which have been mated interspecifically, and not intraspecifically, may lay numbers of sterile eggs (Omori, 1939), and only very rarely is a fertile egg laid (Newberry, 1988; Chapter Six).

A high percentage of sterile eggs laid by mated females therefore reflects failure to re-mate, heat or cold stress, interspecific mating, or some other malfunction of the reproductive system.

Materials and methods

Female bedbugs were collected as described in Section 4.3 above. The eggs of females which died at any stage after capture were scored. Twenty females of each species were re-fed each week for 20 weeks after capture, without further mating, and eggs were scored before each feed. Relevant to this section are 11 mating pairs of C. lectularius maintained as controls for other experiments, and 18 egg-producing female C. hemipterus living in two tubes with an equal number of males, 20 insects in one container and 16 in the other. Feeding and scoring of eggs were carried out weekly, with occasional intervals of two weeks. The ecto-spermeleges of two females were dissected out and placed in lactophenol for 24 hours before being examined under the

microscope for the blackened appearance indicative of interspecific mating in C. lectularius (Walpole, 1988).

Results

See Table 14 for the presentation of data, and Table 15 for its analysis.

In the observations involving repeatedly re-fed females not re-mated since capture, the first batches of totally sterile eggs were produced by two of nine surviving C. hemipterus after seven weeks. One of eight surviving C. lectularius produced all sterile eggs 11 weeks after capture. The last fertile eggs were laid eight and 15 weeks after collection of the insects from Zulu huts by C. hemipterus and C. lectularius respectively, and the last sterile eggs were laid on the 15th and 16th weeks.

One female C. lectularius captured from a hut infested by both species laid a totally sterile batch of 17 eggs. After 24 hours in lactophenol, the ectospermalege of the female did not show the blackened appearance resulting from interspecific mating (Walpole, 1988). Two C. hemipterus females, one from a hut infested by both species, laid ten and 19 eggs in completely sterile batches, and three more of the same species laid high proportions of sterile to fertile eggs (4:21, 4:17; 2:12). These last three bugs were collected in the spring observation described in Section 4.4. Two were discarded before the unusual nature of their

Table 14. Fertile and sterile eggs produced by bedbugs collected from the wild, and bedbugs mated as virgins in the laboratory and maintained for several weeks.

Wild-caught female bedbugs

	C. lectularius	C. hemipterus
No. of bugs	182	121
No. laying only fertile eggs	156	89
No. laying some sterile eggs	26	32
No. of fertile eggs	2513	1587
No. of sterile eggs	53	61
% of sterile eggs	2,07	3,70

Laboratory-reared female bugs

	C. lectularius	C. hemipterus
No. of bugs	22	18
No. of fertile eggs	3220	276
No. of sterile eggs	72	11
% of sterile eggs	2,19	3,80

Table 15. Analysis of fertile and sterile egg production by C. lectularius (L), and C. hemipterus (H) which are wild-caught (wild) or reared in the laboratory (lab). Data presented in Table 14.

Comparison	Chi-squared value (d.f.= 1)	Significance
Fertile: sterile egg ratio in wild and lab L	0,10	N.S.
Fertile: sterile egg ratio in wild and lab H	0,01	N.S.
Fertile: sterile egg ratio in wild L and H	10,20	P<0,01
Fertile: sterile egg ratio in Lab L and H	3,26	N.S.
Fertile: sterile egg ratio in total L and H	14,87	P<0,001
Ratio of wild bugs laying only fertile eggs to those laying some sterile eggs, L and H	6,94	P<0,01
Ratio of bugs laying some sterile eggs to no. of sterile eggs laid, L and H	0,04	N.S.
Above ratio adjusted for the difference in fertile egg output, L and H	0,00	N.S.

eggs was noticed, but the third was dissected and proved to have a black, solid mesospermalege very different from the usual white, gelatinous appearance of this structure.

Discussion

A higher proportion of C. hemipterus eggs are sterile compared with C. lectularius whether they are laid by wild-caught females or insects reared in the laboratory from fifth stage nymphs (though the latter result fails to achieve significance). The discrepancy is caused by more C. hemipterus females laying sterile eggs, rather than by the proportion of sterile eggs being laid differing from C. lectularius.

Cimex lectularius are able to lay fertile eggs for much longer after mating has ceased than C. hemipterus can, a fact also noted by Walpole (1988) in the case of once-mated bugs. However, the hypothesis that lack of re-mating in the wild caused the interspecific difference in sterile egg production cannot be held because constantly mated female C. hemipterus in the present work also laid a high ratio of sterile eggs (Table 14).

It is not known why a C. lectularius female which appeared not to have been interspecifically mated laid a batch of 17 sterile eggs. Either a mating with a male C. hemipterus had occured which did not involve the ecto- or endospermalege (such matings have been observed, though not followed by the

production of eggs), or the female had some defect in its reproductive system.

The mesospermalege of the C. hemipterus female which laid four fertile and 17 sterile eggs resembled that of some wild-caught interspecifically mated C. lectularius females (Walpole & Newberry, 1988). The black hard mass probably results from melanotic cellular encapsulation of sperm (Gotz & Boman, 1985). This implies that some matings between C. hemipterus males and females are incompatible, triggering an immune response in the female to the injected sperm.

The functioning of sperm after entry into the female thus seems to be less efficient in C. hemipterus than in C. lectularius. The sperm remains viable in female C. hemipterus for a shorter period, successfully fertilizes a lower percentage of eggs, and occasionally produces an immune response in the female which reduces fertility considerably.

CHAPTER FIVE

THE SIZE AND COMPOSITION OF BEDBUG INFESTATIONS AND HOST REACTION

5.1 INTRODUCTION

The Cimicidae are able to build up large population densities in the resting places of their hosts. Myers (cited by Usinger, 1966) counted 1333 bugs, excluding the very numerous first stage nymphs, of Oeciacus vicarius in a swallows' nest, and Lee (cited by Usinger, 1966) found 1778 Haematosiphon inodorus in a barn owl's nest. Overal & Wingate (1976) estimated that 55000 Stricticimex antennatus lived in a bat cave, about 220 bugs per host. Lewis (1949) saw "about a thousand" bedbugs (species not mentioned) on one bedstead in the Sudan.

The irritation experienced by a person trying to sleep in an infested room will be proportional to the number of feeding bugs. The size of infestation will also relate to the chances of a person being given an infective dose of a virus, eg., hepatitis B (Jupp et al., 1983) or human immunodeficiency virus (Jupp & Lyons, 1987), the other relevant factor being the life-stage composition of the infestation. Unfed first stage nymphs have no disease organisms in them to transmit, but older nymphs and adults will have a vectorial capacity proportional to the quantity of virus-infected blood they can inbibe (Yanovski & Ogston,

1982). These authors found that adult female C. hemipterus and fifth stage nymphs that became adult females, took more blood than did males at the respective life-stages.

5.2 Cimex lectularius

Bedbug infestations, especially large ones, bring about behaviours in the human host aimed at avoiding irritation or reducing the nuisance. In the present study, the size and composition of C. lectularius infestations were assessed in relation to host numbers and their attempts at bedbug control. Much of this work was presented in Newberry & Jansen (1986), pages 79 to 84 of this thesis, which should be read at this point.

In the above work, the percentage of first stage nymphs which had fed was 11,3 ; 37,3 ; 15,5 ; 41,8 ; 0,0 ; and 49,6 for Huts 1 to 6 respectively. The unusually high percentage of stage one nymphs in Hut 5, none of which had fed, may have been caused by lack of feeding opportunity. Bugs would accumulate without progressing to the second nymphal stage if no blood meal were available. However, no general relationship exists between the percentage of unfed first stage nymphs and the percentage of first stage nymphs in an infestation. The huts 1 to 6 were sampled in the months of December, September, April, July, September and August and no seasonal correlation with life-stage composition is apparent.

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The common bedbug *Cimex lectularius* in African huts

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Abstract

The size and life-stage structure of heavy infestations of *C. lectularius* in Zulu huts were determined. The presence of more than one person in a hut at night, and control measures attempted by hut owners were found not to influence bedbug numbers significantly.

Introduction

Most dwellings in rural KwaZulu (Natal, South Africa) are mud-walled huts with thatched, or sometimes corrugated iron, roofs. These huts provide an abundance of potential harbourages for *Cimex lectularius*, which is often seen in cracks in the mud walls and in furniture.

The medical importance of *C. lectularius* has recently been emphasized by JUPP *et al.* (1983) with the implication of this bedbug in the mechanical transmission of hepatitis B virus. In addition, heavy bedbug infestations can cause iron and haemoglobin deficiencies in their human hosts, debilitation through loss of sleep, and sores when bites become infected from the insects' faecal matter (EBELING, 1975).

Nothing was known about the size or life-stage composition of *C. lectularius* infestations in Zulu huts, nor of the effectiveness of measures taken by inhabitants to control or avoid bugs. The investigation reported here was undertaken in order to provide basic information about this bedbug in KwaZulu.

Materials and Methods

The study was carried out in Zulu huts in the Opansi and Nkundusi areas of KwaZulu. The typical hut is a square or round design with a floor area of 10 to 20 m^2. The mud walls are between 1·5 and 2·0 metres high, with a conical thatch roof supported by poles. Only two of the huts considered had internal partitions. Data supplied by the Weather Bureau, Department of Transport, show that temperatures in the area averaged 25·7°C, 22·7°C, 18·3°C and 21·7°C for each of the four seasons over the years 1979 to 1982. Summer is from December to February, the hottest time of the year.

The insecticide used to assess the size of bedbug infestations was "Insectigas" (registered trademark of Coopers, L2131 Act 36 of 1947). The active ingredient is dichlorvos which is dispensed under pressure from a cylinder containing liquid carbon dioxide to give a highly penetrating aerosol. All windows and other openings were closed or blocked as much as possible, the floor was covered with spray sheets, and a burst of three to six seconds duration was then directed at roof, walls and furniture. The interval between spraying and collection, and between repeated sprayings, varied between experiments. When spraying was conducted at 24-hour intervals, sheets were left down throughout the experiment and collections were made 24 hours after each spraying.

Two methods of collection were used on different occasions to remove dead bugs from the spray sheets. Manual collection was satisfactory for third-stage nymphs and later stages, but missed most of the earlier stages. In order to sample all stages of *C. lectularius*, spray sheets were carefully swept with brushes and the sweepings then examined under a microscope. Occupants were asked how many people spent the night in each hut, and what measures they took to avoid or reduce bedbug infestation. The activities reported included the replastering of mud walls, use of domestic insecticides, and changing of sleeping quarters.

The six huts used for the study of the life-stage composition of *C. lectularius* infestations were chosen when visual evidence suggested the presence of large numbers of bugs. Collection was by sweeping and microscopy.

The study of man-bedbug interaction was carried out in 28 huts belonging to 18 neighbouring Zulu homesteads in the Opansi area of the Ubombo District. Bugs were collected manually from spray sheets.

Bedbugs were identified to species and life-stage by the morphological criteria of USINGER (1966).

Results

Sampling

Post-spraying collections from one hut at irregular intervals up to 48 hours showed *C. lectularius* to be less immediately susceptible to the effects of the insecticide the further it had progressed through its life-stages (Table I). However, between 93% and 97% of each stage (as a percentage of the total catch over 48 hours) was collected in the first 24 hours after spraying. In seven huts tested, between 71% and 79% of all bugs collected from two or three knock-downs were found after the first spray, 16% to 25% after the second and 3% to 9% after a third, irrespective of intervals between spraying and different collection techniques (Tables II).

Life-stage composition of bedbug infestations

Fig. 1 shows that the life-stage composition of *C. lectularius* infestations in three huts, as revealed by two or three knock-downs at 24-hour intervals, is similar to results obtained after only one knock-down. Over 2000 bedbugs were collected in each of four of the six huts considered in Figs. 1 and 2. On average, first-stage nymphs made up about one third of an infestation, with the other four nymphal stages each averaging between 10% and 13%. Adult male and female bugs each averaged 8% of the population. In five of the six huts, fifth stage nymphs amounted to 49% to 62% of the adult *C. lectularius* collected. The corresponding average for the huts considered in Table III is 53%.

Host availability

Regressions were calculated to assess the relationship between the number of people sleeping in each of 28 huts and the size of the corresponding bedbug infestations (Tables III and IV). Negative non-significant correlations were found for either

Table I—The cumulative knockdown of each bedbug life-stage, with percentages in parentheses: (i) at intervals after spraying one hut with dichlorvos and (ii) the collection made 48 hours after a subsequent second dichlorvos treatment two weeks later. Collections made by sweeping and low-power microscopy

		Time interval between spraying and collection				48 hours after second spray, two weeks later
		2 hours	6 hours	24 hours	48 hours	
(Nymphal stages) (adults)	♂	126 (35·7)	259 (73·4)	338 (95·8)	353 (100)	133
	♀	96 (40·5)	152 (64·1)	228 (96·2)	237 (100)	80
	V	153 (52·4)	205 (70·2)	272 (93·2)	292 (100)	67
	IV	216 (69·7)	258 (83·2)	289 (93·2)	310 (100)	72
	III	422 (76·7)	466 (84·7)	530 (96·4)	550 (100)	46
	II	391 (83·9)	424 (91·0)	447 (95·9)	466 (100)	34
	I	547 (85·7)	591 (92·6)	600 (94·0)	638 (100)	453

Table II—Collections of bedbugs from spray sheets in seven huts at various intervals after spraying with dichlorvos

Date of spraying	Interval between spraying and collection	Method of collection	Number (%) of bugs collected
HUT A			
15.7.82	6 hours	Visual	1308 (77·9)
11.8.82	6 hours	search	312 (18·6)
25.8.82	6 hours	of sheets	58 (3·4)
HUT B			
15.7.82	6 hours	Visual	339 (71·2)
11.8.82	6 hours	search	120 (25·2)
25.8.82	6 hours	of sheets	17 (3·6)
HUT C			
19.4.83	24 hours	Visual	73 (78·5)
20.4.83	24 hours	search	15 (16·1)
21.4.83	24 hours	of sheets	5 (5·4)
HUT D			
15.12.82	24 hours	Sweeping of	3757 (76·6)
16.12.82	24 hours	spray sheets	1149 (23·4)
HUT E			
19.4.83	24 hours	Sweeping	404 (74·5)
20.4.83	24 hours	of	91 (16·8)
21.4.83	24 hours	spray sheets	47 (8·7)
HUT F			
28.9.83	24 hours	Sweeping	1068 (70·5)
29.9.83	24 hours	of	347 (22·9)
30.9.83	24 hours	spray sheets	100 (6·6)
HUT G			
11.8.82	48 hours	Sweeping of	2790 (75·9)
24.8.82	48 hours	spray sheets	885 (24·1)

number of adult bugs or all stages ($r = 0{\cdot}03$ and $-0{\cdot}25$ respectively; $P>0{\cdot}05$). When only the nine huts are considered in which no domestic insecticides had been used, correlations are still not significant ($r = -0{\cdot}50$ and $-0{\cdot}61$; $P>0{\cdot}05$).

Replastering the mud walls

18 of 28 huts had been replastered with mud after the annual anti-malaria spraying programme, when DDT is sprayed on the inside walls and roofs of all buildings in the area. Hut occupants replaster in order to cover up the DDT deposit which they believe irritates the bugs and increases biting, and to reduce the number of refuges available for bugs (NEWBERRY *et al.*, 1984). However, the mean knock-down catches of bedbugs from replastered and non-replastered huts were very similar, at 236 and 244 respectively. Seven of the 10 non-replastered huts, and 11 of the 28 replastered huts, had been previously sporadically treated with domestic insecticides by the occupants.

Use of domestic insecticides

The 18 huts in which domestic insecticides were reportedly used had an average of 195 bugs per hut, compared with 318 bugs per hut in the ten dwellings where no insecticide was used. The difference between the means is not significant at the 5% level.

Alternation of sleeping quarters

Two instances were encountered of changed sleeping quarters in response to bedbug attack. One family alternated between two huts; the one currently

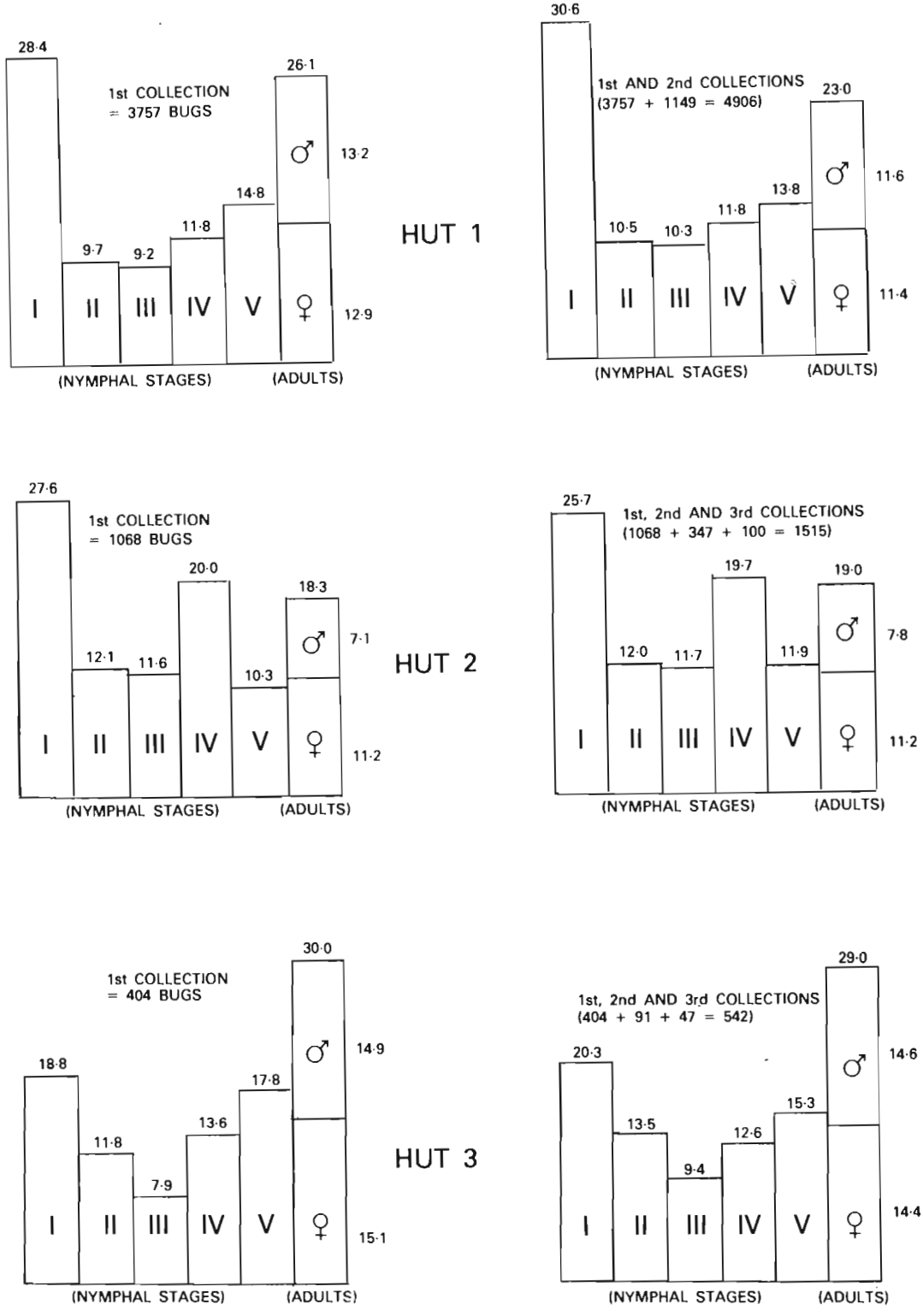

Fig. 1. Percentage life-stage composition of bedbug infestations in 3 huts sampled by knock-down with dichlorvos at two or three 24-hour intervals, each collection made 24 hours after spraying by sweeping and low power microscopy.

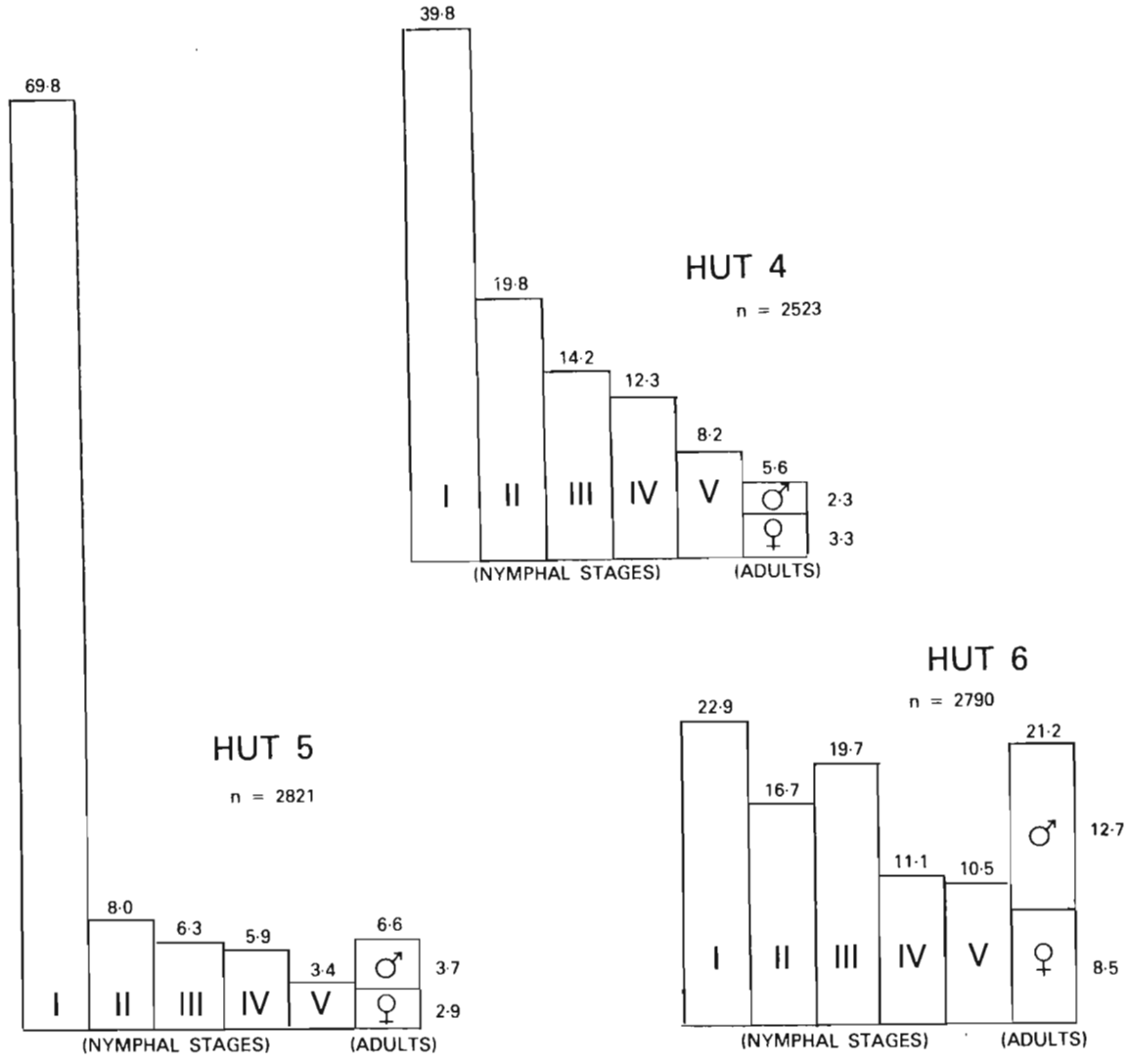

Fig. 2 Percentage life-stage composition of bedbug infestations in 3 huts sampled by a single dichlorvos knock-down; collection 24 hours post-spraying, by sweeping and low-power microscopy.

Table III—Average number of bedbugs per hut found in 23 huts, 24 hours after knock-down, in relation to occupancy by one adult and up to five children. Bugs were manually collected from spray sheets

Occupants/hut	Number of huts (Total = 23)	Adult bugs: average catch per hut	Adults and nymphs: catch per hut		
			Average catch	Maximum catch	Minimum catch
1 Adult	6	40	258	1225	5
1 Adult + 1 child	2	190	686	1244	128
1 Adult + 2 children	2	95	205	338	72
1 Adult + 3 children	3	153	241	546	25
1 Adult + 4 children	4	63	186	709	0
1 Adult + 5 children	6	112	184	522	51

Table IV—Numbers of bedbugs found per hut 24 hours after knock-down in five huts used by adults and/or children. Bugs were manually collected from spray sheets

Occupants/huts	Number of adult bugs	Number of adults and nymphs
2 children	214	487
7 children	4	5
4 adults	27	55
2 adults + 4 children	39	108
2 adults + 5 children	35	98

occupied and that temporarily unused yielded 22 and 556 bugs respectively. These two huts were excluded from the rest of the study because of the periodic unavailability of hosts to the bedbugs. Another man alternated between the four rooms of his hut for sleeping purposes; each room yielded between 13 and 36 bugs on knock-down. The rooms were in a straight sequence, each about 3 m long.

Discussion

Sampling

Table I shows that most bugs are collected in the first 24 hours after spraying with dichlorvos, relatively few are knocked down between 24 and 48 hours after spraying and the catch after 48 hours is probably minimal. Collections made up to 48 hours will therefore approximate to the total knock-down.

If dichlorvos knock-downs in a mud hut were 70% effective, one would get 70%, 21% and 6·3% of a bedbug infestation knocked-down by three consecutive treatments. This corresponds well with the results recorded in Table II. Correcting for loss when a 48-hour catch is not made after the last spraying, it is estimated that a three-spray programme at 24-hour intervals, with collections 24 hours after spraying, will collect about 97% of all bugs in a hut. A two-spray programme will collect about 90%, and a single spray about 66%. On these assumptions the six huts considered in Figs. 1 and 2 thus had populations of about 558, 5451, 1561, 3822, 4227 and 4274 bedbugs respectively.

The consistency of the results recorded in Table II occurs despite variation in collecting method and intervals between spraying. This is because all collections except one made in huts at intervals of a few weeks, used the manual method of sampling. Bugs which had emerged from eggs laid after spraying were in early nymphal stages and therefore usually overlooked, so only bugs present in the original population were sampled thoroughly. The exception is hut G in Table III, where collection was by sweeping spray sheets 48 hours after each spraying, there being two weeks between the first and second spraying. Stage one nymphs formed a far larger proportion of the second collection (51·2%) compared with the percentage in the first (22·9%), which suggests that eggs laid by the pre-spraying population had survived and hatched. If only stage three and older bugs are considered, 80·9% were collected by the first spray and 19·1% by the second.

Life-stage composition

The numbers of the different life-stages found in post-spraying collections depends on the accuracy of discovery of dead bugs, susceptibility to insecticide, and the duration and survival rate of each life-stage. Dichlorvos has a flushing effect, and all bugs falling on to the sheets are collected when the sweeping method is used. The comparisons of collections made after one and more consecutive sprayings (Fig. 1) suggest that all life-stages were similarly affected. Were this not so, the more susceptible stages would be most heavily represented in the collection after the first spray. The life-stage structure of collections can therefore be interpreted in terms of the biology of the infestations.

At the temperatures encountered in this field study, the durations of the first four nymphal stages are similar, each being about three quarters that of the fifth nymphal stage (JOHNSON, 1941). Differences in the numbers of bugs in the various life-stages must therefore be explained in terms of the environment interacting with the population of bugs.

The collections from the six huts studied all had a high percentage of first stage nymphs. This would be expected in a new population rapidly growing (JOHNSON, 1941), but all infestations studied were of long standing. The explanation is probably that a large number of first stage nymphs fail to develop to stage two. As first stage nymphs can survive weeks without food (JOHNSON, 1941) it is unlikely that unavailability of food is the cause of heavy mortality. Possibly the bug's ability to detect its host for the first blood meal of its life is less efficient at the first nymphal stage. Whatever the problems to survival, they appear to be solved in the second nymphal stages, for five of the six collections studied (Fig.s 1 and 2) show little decline in numbers over the later nymphal stages. The findings that stage five nymphs number about half that of adults in the same infestations must mean, assuming equal mortality rates, that adults usually live about twice as long as the duration of the fifth nymphal stage. Adults can make a relatively large (29%) or small (6%) proportion of the total population, and so cannot be used as an accurate index of population size.

Interaction between C. lectularius *and man*

The number of hosts in a hut, above one, had no discernible effect on bedbug infestation size. An extreme example of this in the present study (Tables III and IV) is the hut occupied by one man which yielded 1225 bugs in knock-down, compared to the five bugs collected in a hut used at night by seven children. This suggests that either one person in a hut is all that is needed to support a very large bedbug infestation, or that other more powerful factors are masking the effects of host availability. Replastering of mud walls had no demonstrable effect on bedbug numbers, which suggests that the number of potential harbourages is not reduced to a critical level.

The mean number of bugs in huts where use of domestic insecticides was reported, was much lower than in huts where no insecticide was used. However, the variance in both samples is so great that the difference between the means is not significant. In general, it can therefore be stated that domestic insecticides are not used effectively.

Conclusion

Heavy *Cimex lectularius* infestations occur in many dwellings in rural KwaZulu and the measures taken by hut occupants, though of limited effectiveness, reflect their disturbance by the bugs. It is not clear what factors regulate the size of bedbug infestations in the mud-hut environment.

"Insectigas" (registered trademark of Coopers, L2131 Act 36 of 1947) provides a convenient and effective method to sample bedbug infestations, and estimates of total numbers in a hut can be made from a collection made 24 hours after a single spray knock-down.

Acknowledgements

We are grateful to Drs. C. F. Hansford and P. G. Jupp for detailed criticism of the draft; to Mr. A. R. Pope of Coopers (SA) (Pty) Ltd., who provided the knock-down insecticide; and the Director General for the Department of Health and Welfare, Pretoria, for permission to publish.

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The life-stage compositions of C. lectularius infestations (Figs. 1 and 2 (especially Hut 6) of Newberry & Jansen (1986), pages 79 to 84 of this thesis) in Zulu huts resemble the findings of Mellanby (1939) (Fig. 3 (top)) who reported on an infestation in a heated room where laboratory rats were kept. Johnson (1941) stated that Mellanby's (1939) data fitted well with his theoretical model of the growth of a C. lectularius infestation. However, the proportions of the various nymphal and adult stages vary to such an extent from week to week in Johnson's (1941) model that any infestation is likely to fit some stage of his theoretical construction. Certainly, the second to fifth stage nymphs are more equally represented in Mellanby's (1939) rat house and the Zulu huts reported above, than in Johnson's (1941) model in which nymphal stages are subject to violent fluctuations in numbers. This is probably because in nature, eggs and nymphs do not all hatch and moult in unison as they did in Johnson's (1941) model. None of the life-stage histograms for C. lectularius (Figs. 1 & 2, Newberry & Jansen, 1986) resemble that of Stricticimex antennatus (Fig. 3 (bottom), page 87), in that the percentage constitution of the first stage nymphs of the latter species is very low (14%).

Titschack (cited by Usinger, 1966) estimated the average amount of blood taken at a single feeding by each life-stage of C. lectularius. It is therefore possible to calculate how much blood a person sleeping in Hut 1 (Newberry & Jansen, 1986) would lose if all the bugs fed on him. Following

Johnson (1941) it appears that about 15 g of blood would be taken, perhaps every five days so the total over one year would be about 1 litre. This would not in itself be of consequence to a healthy adult, though might act as a drain in the vitality of a sick child. Of far more importance is the irritation caused by so many bites, the chances of secondary infection, and the mechanical transmission of viruses.

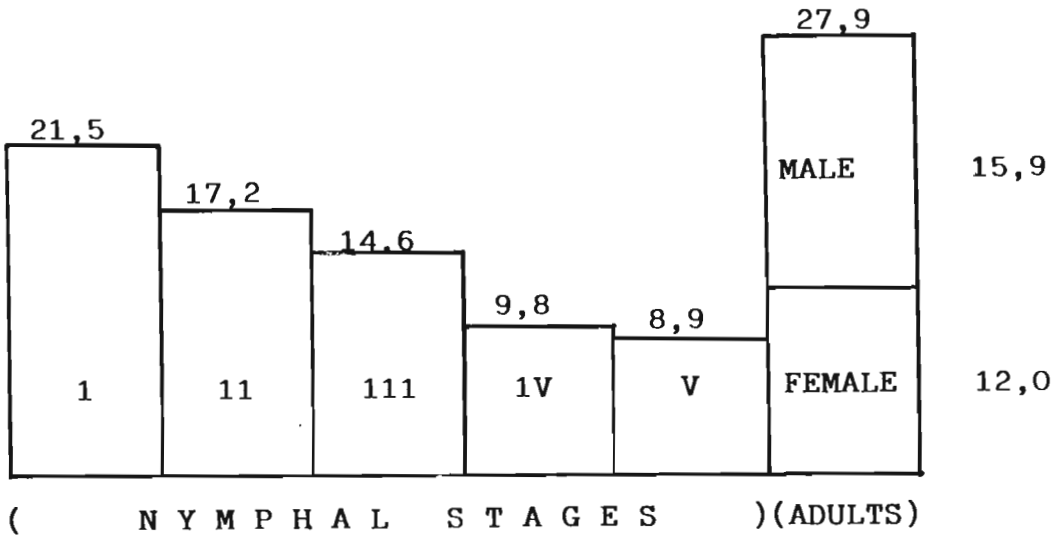
5.3 Cimex hemipterus

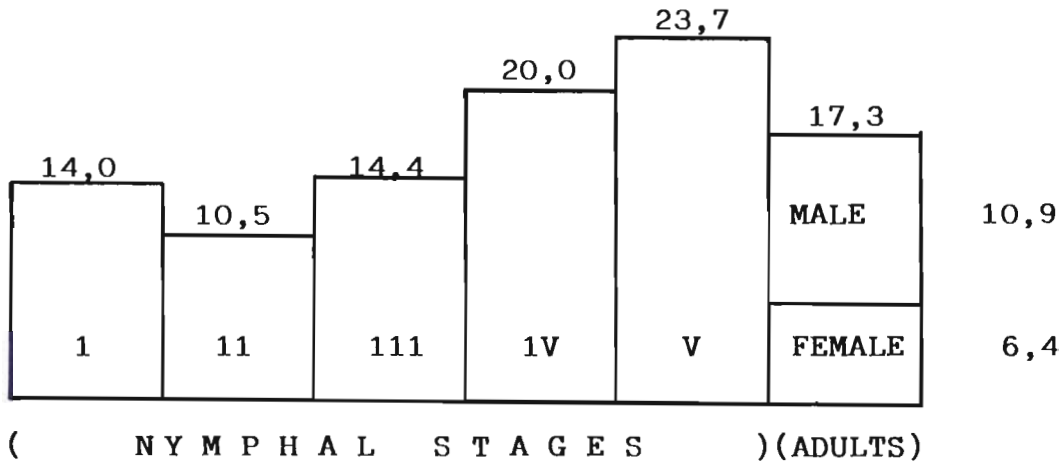
Materials and methods

Cimex hemipterus bugs were collected from spray sheets covering the floors of 16 Zulu huts which had been sprayed with fenitrothion 24 hours previously (Chapter Eight). Collections from 21 huts infested with C. lectularius were similarly made. Two collections of C. hemipterus, in December 1985 and January 1986, were made using Insectigas knockdown (active ingredient dichlorvos, 93 g per kg) and sweeping of spray sheets as described in Newberry & Jansen (1986) on page 79 of this thesis. Nymphal stages were identified by head-width, other morphological criteria being used for borderline cases (Chapter Two).

Results

The huts sprayed with fenitrothion yielded an average of 90,9 adult and nymphal C. hemipterus (range: 2 - 449) as compared to an average of 91,5 C. lectularius (range: 1 -


21,5
17,2
14,6
9,8
8,9
27,9
MALE
15,9
1
11
111
1V
V
FEMALE
12,0
(NYMPHAL STAGES)(ADULTS)

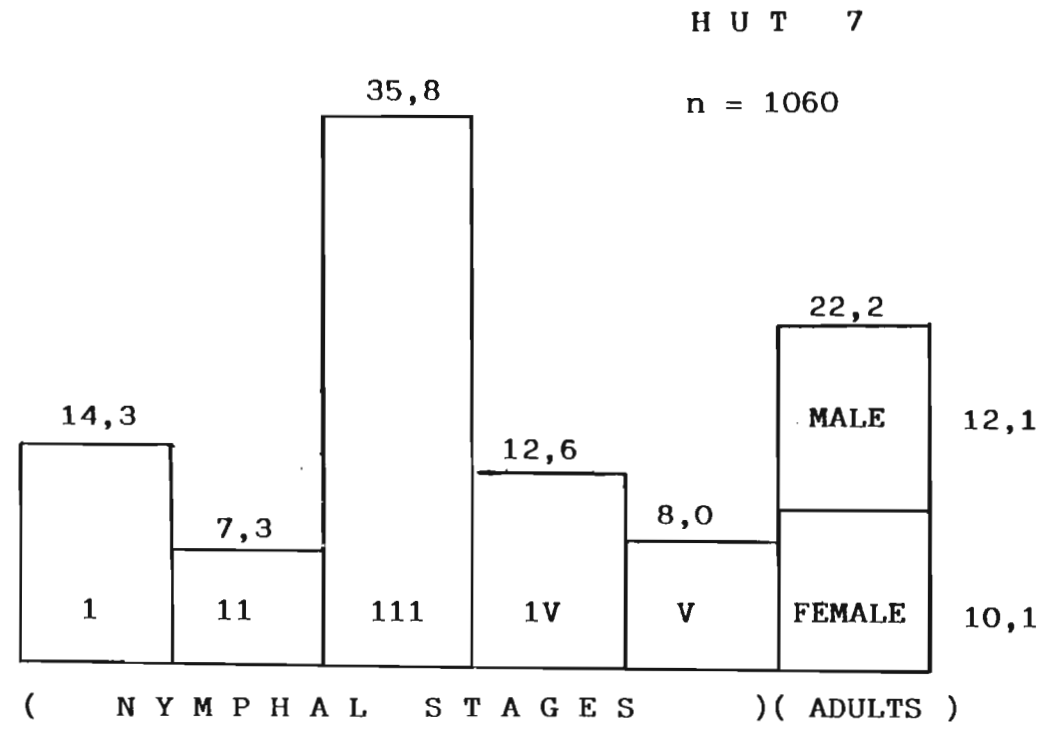

14,0
10,5
14,4
20,0
23,7
17,3
MALE
10,9
1
11
111
1V
V
FEMALE
6,4
(NYMPHAL STAGES)(ADULTS)

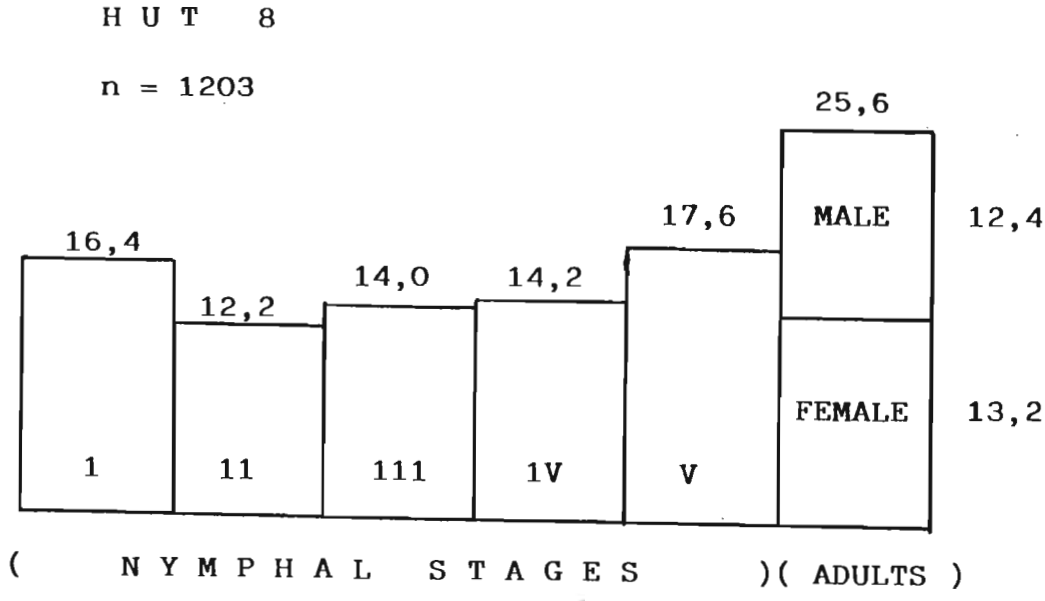
403). The difference between the means of the two species is not significant (t = 0,02; d.f. = 35; N.S.). The Insectigas knockdowns yielded 1060 and 1203 C. hemipterus adults and nymphs from huts 7 and 8 respectively (Fig. 4), the percentage of fed first stage nymphs being 6,6 and 54,8.

Discussion

Although at the southernmost tip of its known distribution (Newberry et al., 1987), C. hemipterus infests human habitations in as large numbers as does C. lectularius.

In huts 7 and 8 (Fig. 4), first stage nymphs made up less than 17% of the infestations, whereas this stage constituted between 20 and 70% of C. lectularius infestations (Figs. 1 and 2, Newberry & Jansen, 1986). Many more huts harbouring these species would need to be sampled at different times of the year to see if there are any consistent differences in life-stage composition. If real, the lower percentage of stage one nymphs in C. hemipterus could mean a higher survival rate, in that fewer die before reaching stage two, or a recent drop in egg production. The rather erratic histogram profile of hut 7 perhaps reflects host availability, the smooth profile of hut 8 indicating more stable conditions, but a longitudinal study would be necessary to substantiate such hypotheses.


H U T 7
n = 1060
35,8
14,3
12,6
22,2
8,0
7,3
MALE
12,1
1
11
111
1V
V
FEMALE
10,1
(N Y M P H A L S T A G E S)(ADULTS)


H U T 8
n = 1203
25,6
17,6
MALE
12,4
16,4
14,0
14,2
12,2
FEMALE
13,2
1
11
111
1V
V
(N Y M P H A L S T A G E S)(ADULTS)

CHAPTER SIX

THE OCCURRENCE AND EFFECTS OF INTERSPECIFIC MATING BETWEEN *Cimex lectularius* AND *Cimex hemipterus*

6.1 INTRODUCTION

Insemination in the Cimicidae is traumatic. The male punctures the body wall of the female with its copulatory organ, the paramere, and injects sperm into the body cavity. The site of penetration is the spermalege which is composed of an external groove (the ectospermalege) and an internal whitish, hemispherical tissue (the endospermalege) into which the sperm is deposited. The sperm then has to migrate across the body cavity and penetrate the internal reproductive organs to gain access to the ovarioles (Carayon, 1966).

Male cimicids do not restrict their mating activities to females of the same species. Rivnay (cited by Usinger, 1966) found male *C. lectularius* would copulate with other males, dead females, and a bug-shaped piece of cork. Rao (1972) also noted homosexual activity among male *C. lectularius*, insemination always having fatal results to the recipient. Davis (1966) mentioned that male *Afrocimex* have a spermalege and are routinely mated by other males, though without any reproductive consequences because no other female organs are present.

The degree to which homosexual mating occurs in the Cimicidae possibly indicates that specific-mate recognition (Paterson, 1985) is poorly developed. Though some degree of positive assortative mating between C. lectularius and C. columbarius is apparent (see Chapter Seven) interspecific insemination does frequently take place in the laboratory even in the presence of an equal number of conspecific mates (Ueshima, 1964). Male C. hemipterus also readily mate with female C. lectularius (Omori, 1939), the reciprocal mating being less spontaneous (Walpole, 1988a), sometimes taking place more than 24 hours after cohabitation as compared to up to 4 hours for the former cross.

6.1.1 Interspecific mating among Hesperocimex species and between the bedbugs

Ryckman & Ueshima (1964) cross-mated Hesperocimex cochimiensis, H. sonorensis and H. coloradensis and found not only varying degrees of fertility but also that interspecific mating could be lethal to the female. Dying females were paralysed and developed a progressively enlarging dark area around the spermalege. After death, females had swollen black abdomens and sometimes a dried plug of serum extruded from the spermalege. Ryckman & Ueshima (1964) attributed these deaths to excessive trauma caused by interspecific mating followed by disease produced by invading microorganisms. Similarities to other research findings exist here in that paralysis and death were the

fate of all homosexually mated C. lectularius males (Rao, 1972), and Omori (1939) stated that interspecific mating with C. hemipterus males shortened the life span of C. lectularius females (discussed below). Walpole (1988b) reported that the ectospermalege of C. lectularius became filled with a dark brown mass following a single interspecific mating with C. hemipterus, and Walpole & Newberry (1988) noticed that some female C. lectularius captured in huts infested by both bedbug species had blackened mesospermaleges in addition to dark ectospermaleges. Rather than being due to disease organisms invading the abdomen following interspecific mating (Ryckman & Ueshima, 1964) the darkening of the spermalege noticed in C. lectularius females, with more extensive tissue damage in Hesperocimex, is probably due to melanotic cellular encapsulation of the alien sperm by the female immune system (Gotz & Boman, 1985), and death results from the toxic effect of the foreign sperm.

6.1.2 Omori's (1939) hypothesis of species replacement brought about by interspecific mating

Omori (1939) conducted a series of ten observations in which one or two bedbugs of each sex and species were placed together in tubes and scored for several weeks for mortality and egg production. Omori concluded from his results that cohabitation with C. hemipterus often greatly reduced the life span of female C. lectularius, and always shortened the

time during which fertile eggs were laid, these effects becoming more pronounced with increasing temperature. He stated that no such damage was done to C. hemipterus females under the same circumstances, and suggested that it was the C. hemipterus sperm that caused the adverse effects on the C. lectularius females.

Omori's (1939) data is consistent with his hypothesis but falls short of proving it because no single species controls were monitored for comparison. Male C. lectularius also did not survive long (x = 82 days) in the trials where C. hemipterus males (x = 143 days) were not accidently lost, raising the possibility that C. lectularius females (x = 52 days) were shorter lived than C. hemipterus females (x = 118 days) for some reason other than interspecific mating. The ten observations were carried out at three different temperature regimes (four at 27 degrees C, four at outdoor temperatures 15 - 28 degrees C, and two at room temperatures 19 - 29 degrees C), which unnecessarily complicates the research design. There are errors in the calculations of longevity in Tables 1, 4, 5 and 6 of Omori (1939).

Omori (1941) presented the above data again in a 174 page work with other findings that can serve as a guide to the meaning of his results on interspecific cohabitation. He recorded that at 27 degrees C C. lectularius females cohabiting with conspecific males lived an average of 105 days as compared to 111 days for female C. hemipterus (males

averaged 158 and 178 days respectively). Comparison with the data given in the previous paragraph shows that reduction in life span appears to have occurred in both sexes of C. lectularius in Omori's (1939) cohabitation trials. However, his second point concerning the suppression of the production of fertile eggs does receive clear support. Omori (1941) recorded that at 27 degrees C the egg laying period of C. lectularius in single species trials ranged between 82 and 116 days (n = 7; x = 91,6), while C. hemipterus females had egg laying periods of between 36 and 98 days (n = 6; x = 74,7). In Omori (1939), the 11 C. hemipterus females in eight comparable trials where both species were present produced 2199 fertile eggs (x = 199,9) compared to the 583 (x = 53,0) laid by C. lectularius. Fertile eggs were therefore laid in mixed species trials at a ratio of 1 : 0,27 in favour of C. hemipterus, whereas in single species trials at between 22 and 30 degrees C the ratio was 1 : 1,4 (Omori, 1941).

Omori (1941) made a further discovery in that if the males of C. lectularius and C. hemipterus cohabited alternately with C. lectularius females at 23,4 degrees C the percentage of sterile eggs laid was 4 when the male C. lectularius was used first, but 71,4 when the other species preceded. Experiments at 22 and 27 degrees C resulted in 2,9 and 12,2% of eggs being sterile when conspecific cohabitation occurred first, and 90,1 and 91,7% sterility when the interspecific cross preceded. Eggs produced by C. hemipterus females were

not significantly affected in two of the trials by which species of male was used first, though sterility rose from 4,6% to 20,7% in the trial at 22 degrees C. As 4,3% of eggs laid by C. hemipterus females mated only by their own species were sterile (Omori, 1941) this could be a biologically significant finding, but the low numbers used in Omori's trials (n = 6) and the variability shown in the production of sterile eggs by female bedbugs (see Chapter Four) leave room for doubt.

Omori's (1939, 1941) results therefore suggest that C. lectularius females, and perhaps males too, tend to die sooner when cohabiting with C. hemipterus than when living with conspecific bugs only. The reproductive ability of female C. lectularius is further affected by a reduced egg laying interval and a higher percentage of sterile eggs if mated by C. hemipterus first when males are alternated over a period of weeks. Omori's (1939, 1941) data do not suggest, however, that the lifespan of C. hemipterus is affected by cohabitation with C. lectularius, and the former species produced large numbers of fertile eggs over several weeks in mixed species trials. If any adverse effects on the fertility of C. hemipterus are brought about by interspecific cohabitation, they are of considerably less consequence than those suffered by C. lectularius.

Using these laboratory results, Omori (1939, 1941) provided a basis for the suggestion (which he attributed to Rulter

(1923) but without entering the reference in his bibliography so the article cannot be traced) that C. lectularius was not found in some tropical areas because of the presence of C. hemipterus. Omori's (1939) hypothesis is supported by Davis (1966) in that Davis found the seminal fluid of C. hemipterus to be toxic to C. lectularius (no further details were given).

6.2 TESTING OMORI'S (1939) HYPOTHESIS

The validity of Omori's hypothesis depends both on the severity of the effects of interspecific mating, and how often such mating occurs in nature. Using the darkened ectospermalege (Walpole, 1988b) as a marker, Walpole & Newberry (1988) investigated the occurrence of this phenomenon in two huts infested by both bedbug species with C. hemipterus much the more numerous. This work is presented on Pages 99 to 102 of this thesis and should be read at this point. Newberry (in press) also investigated interspecific mating in the laboratory, repeating Omori's (1939) work on a scientific basis, extending field observations to consider huts with varying proportions of the two species, and observing egg production of C. lectularius taken from these huts (pages 103 to 124 of this thesis). Data for the analyses presented in Table 4 of Newberry (in press) appear in Table 16 of this thesis (pages 125 to 130).

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A field study of mating between two species of bedbug in northern KwaZulu, South Africa

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ABSTRACT. The interspecific cross-mating of female *Cimex lectularius* Linnaeus with male *Cimex hemipterus* (Fabricius) results in a visible mark developing in the female ectospermalege. This mark has been used to record the frequency of mating between female *C.lectularius* and male *C.hemipterus* in sympatric wild populations in a DDT-sprayed area of northern KwaZulu, South Africa. A high percentage (11/16=69%) of *C.lectularius* females were marked, showing that these two species mate in nature.

Key words. *Cimex hemipterus*, *Cimex lectularius*, bedbugs, interspecific mating, hybridization, KwaZulu, South Africa.

Introduction

Records of two domestic species of anthropophilic bedbug, *Cimex lectularius* Linnaeus and *Cimex hemipterus* (Fabricius), show that they occasionally occur in sympatry (Usinger, 1966), although their distributions are mostly allopatric, *C.lectularius* mainly in cooler environments and *C.hemipterus* in tropical situations. Laboratory cross-mating studies between the two species (Omori, 1939) showed that the interspecific cross of *C.lectularius* females with *C.hemipterus* males resulted in the production of infertile eggs and the reduction of female longevity. He suggested, therefore, that the absence of *C.lectularius* from parts of the world where *C.hemipterus* was common, was due to the adverse effects of cross-mating on *C.lectularius* females.

Newberry *et al.* (1987) reported that *C.lectularius* and *C.hemipterus* occur in sympatry in DDT-sprayed houses in KwaZulu, north of Lake St Lucia, South Africa (28° 15′ S). They also found that some of the houses infested by both bedbug species, yielded only *C.hemipterus* on a second sampling a few months later. Omori's hypothesis, as related in Usinger (1966), was suggested as a possible explanation, with the proviso that frequent interspecific mating in the wild would have to occur for *C.lectularius* populations to be eradicated.

Walpole (1988) found that *C.lectularius* females that had been mated once by *C.hemipterus* males developed a dark mass in the ectospermalege. This mass appeared to be permanent, and enabled interspecifically mated *C.lectularius* females to be easily distinguished

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from those that were either virgins or conspecifically mated, i.e. scarred.

This paper presents the results of using the dark mass as a marker to observe the frequency of interspecific mating between female *C.lectularius* and male *C.hemipterus* in houses infested by both species, in northern KwaZulu, South Africa.

Materials and Methods

Both species of bedbug were found in two mud-walled, DDT-sprayed houses used as human habitations in the Nkundusi/Madweleni area of northern KwaZulu (28° 15′ S, 32° 23′ E). A total of nine collections were made in the two houses during October and November 1986.

By way of a control, female *C.lectularius* were collected in November, from two DDT-sprayed houses in the Nkundusi district which in previous collections had yielded only *C.lectularius*.

Bedbugs were collected individually by hand and the species were identified using external morphological features, especially the shape of the pronotum and abdomen, body size and, to a lesser extent, the coloration. Any *C.lectularius* females collected were placed in individual tubes. Male and female *C.hemipterus* and male *C.lectularius* were sorted into one container for each group.

Thermohygrograph recordings were take inside the house in Madweleni, during the November collection. Readings were as follows: average 24 h maximum and minimum temperatures were 23°C and 19°C. The average 24 h maximum and minimum humidities were 87% and 73% r.h. respectively.

Females collected from the house in Madweleni were examined under a stereo dissecting microscope for the presence of sperm in the ectospermalege or the seminal conceptacles, which are signs of recent mating (Walpole, 1988). Abdomen distention, gut colour and content were used to estimate, visually, the nutritional status of all the females collected from the house in Madweleni (Table 1). Nutritional status was recorded as either: (i) fully distended with signs of a recent bloodmeal, i.e. red coloration; (ii) partly distended with a partially digested bloodmeal, dark brown in colour; or (iii) unfed, i.e. no distention and little bloodmeal left.

Female *C.lectularius* were examined for conspecific or interspecific mating under a stereo dissecting microscope and then cleared in lactophenol to confirm the result (Walpole, 1988).

Results and Discussion

The numbers and the mating and nutritional status of live female bedbugs collected from sympatric populations are given in Table 1.

The Nkundusi house was sampled three times in October 1986. On the first two occasions, six female and four male *C.lectularius* were found. On the third collection two female and three male *C.lectularius* and twenty-six female and forty-eight male *C.hemipterus* were found. Only five of the *C.lectularius* females were isolated into individual tubes on capture and therefore suitable for cross-mating studies.

TABLE 1. Numbers of live female bedbugs and their mating status, collected sympatrically in DDT-sprayed houses in the Madweleni and Nkundusi districts of KwaZulu. The nutritional status is given separately for females collected at Madweleni.

	C.hemipterus	*C.lectularius*
No. collected	68	16
No. with recent sperm transfer	18	0
No. conspecific matings	Indeterminate	3
No. interspecific matings	Indeterminate	11
No. virgins	Unknown*	2
Nutritional state		
Fully distended	2	0
Partly distended	28	0
Unfed	4	16

* Females were not examined.

The house in Madweleni was sampled twice in October and four times in November 1986. A total of fourteen female and nineteen male *C.lectularius* were collected. Three of the females were dead and therefore not included in the results. Both species were found together in five of the six collections, totalling ten female and fifteen male *C.lectularius* and forty-two female and twenty-eight male *C.hemipterus*.

Thirty-two female *C.lectularius* were collected from the two 'control' houses. Thirteen of these females were dissected and cleared in lactophenol and the other nineteen were pinned. Of the dissected females, twelve had been conspecifically mated and one was a virgin. The pinned specimens were either virgins or conspecifically mated. No females that had been marked by *C.hemipterus* males were collected.

The morphology of the dissected ectospermalege of the marked wild-caught females was similar to that observed during laboratory studies (Walpole, 1988). Prior to dissection, the size and shape of the dark mass around the paragenital sinus varied amongst the wild-caught females (Figs 1a and 2). This variation had not been observed previously in females that had been subjected to a single interspecific mating (Fig. 1b). On dissection, the mass was found to be the mesospermalege containing dark material which was easily removed with dissecting needles, leaving the marked ectospermalege intact.

All the *C.lectularius* females collected from the house in Madweleni were unfed and not recently mated, whereas *C.hemipterus* females showed a variety of nutritional and mating states (Table 1).

The high percentage of interspecifically mated female *C.lectularius* (69%) collected from double infested DDT-sprayed houses (Table 1) was unexpected. While natural interspecific

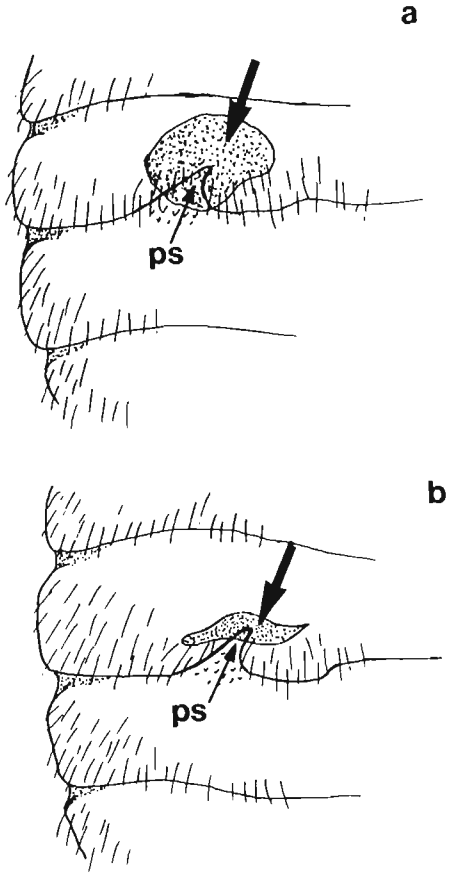

FIG. 1. Diagram showing the difference in the external appearance of the mark (thick arrow) around the paragenital sinus (ps) of *C.lectularius* females prior to dissection of (a) wild-caught females, and (b) laboratory females that had been mated once with a *C.hemipterus* male (not to scale).

FIG. 2. Photograph of the external appearance of the darkened mesospermalege (arrow) seen in some of the wild-caught *C.lectularius* females (×18).

mating is rare (Mayr, 1963; Paterson, 1964; Dobzhansky & Powell, 1975), when it does occur in nature, the frequency is usually much lower than under laboratory conditions (e.g. Norval & Sutherst, 1986).

The results presented here showed that, at least in DDT-sprayed dwellings and where *C.hemipterus* predominated, most of the *C.lectularius* females collected had been mated interspecifically. Assuming the deleterious effects, i.e. reduction of longevity (Omori, 1939) and sterility (Omori, 1939; Walpole, 1988; Newberry, 1988), of interspecifically mated *C.lectularius* females, it would seem that interspecific mating could be a factor in limiting the distribution of *C.lectularius* in the area of sympatry studied.

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The effects on domestic infestations of bedbugs *Cimex lectularius* of interspecific mating with *C. hemipterus*

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ABSTRACT. Laboratory trials indicate that the longevity and fertile egg production of female *C. lectularius* are reduced by mating with male *C. hemipterus*. Interspecific mating takes place freely in the laboratory, even when many female *C. hemipterus* are available. Interspecifically mated female *C. lectularius* have been found in human domiciles in KwaZulu, a correlation existing between the percentage of *C. lectularius* females which are interspecifically mated and the predominance of *C. hemipterus* in the bedbug infestation. A high percentage of female *C. lectularius* lay only sterile eggs when *C. hemipterus* constitute over 75% of the infestation, but very few show this behaviour when *C. lectularius* is the most numerous species.

Key words. *Cimex hemipterus*, *Cimex lectularius*, interspecific mating.

Introduction

Omori (1939) proposed that the island of Formosa (Taiwan) was free from infestation with the Common Bedbug, Cimex lectularius Linnaeus because the resident Tropical Bedbug, C. hemipterus (Fabricius), prevented the former species from establishing itself. The mechanism he suggested was that when female C. lectularius mated with male C. hemipterus, very often the lifespan of the female was considerably shortened, and its normal ability to produce fertile eggs impaired.

Omori based this hypothesis on his laboratory cross-mating studies involving up to eight insects per replicate, two of each sex and species. Although his data indicated the trends outlined in the preceding paragraph, his basic observations did not have control series and thus failed to unequivocally prove his contentions. The interaction of the two species in numbers above two of each species and sex was also not investigated by Omori, though large numbers of insects are present in natural infestations.

Omori's (1939) hypothesis assumes that interspecific mating occurs in the field, and to an extent that C. lectularius females are damaged. Data supporting this assumption are included in Newberry et al. (1987) and Newberry & Mchunu (1989), who recorded displacement of C. lectularius by C. hemipterus in five human habitations, and no instances of

the reverse process. In addition, Walpole & Newberry (1988) found that 69% of female C. lectularius in two huts where C. hemipterus predominated had been interspecifically mated.

This study is intended to test Omori's (1939) findings on an experimental basis, and to extend his laboratory observations to include the cohabitation of larger numbers of bedbugs of both species. In the field, the observations of Walpole & Newberry (1988) are extended to include huts where C. lectularius outnumbered C. hemipterus, and to assess the impact of interspecific mating on these wild infestations of C. lectularius.

Materials & Methods

Collections from human habitations containing both species of bedbug

Seven huts in northern KwaZulu containing mixed infestations were sampled between October 1986 to April 1987. Five of the huts were in the Nkundusi/Madwaleni area (centering on 28° 10'S; 32° 20'E), one at Manaba (27° 15'S; 32° 15'E) and another at Mamfene (27° 23'S; 32° 10'E). At Manaba (Hut 4, Table 1) collections were by insecticide knockdown. In all other huts, bugs were collected one at a time from harbourages in walls and furniture and kept in individual tubes. Scoring into species and sex was done using morphological characters (Usinger, 1966). Female C. lectularius were retained in their tubes and the eggs they

laid were counted and scored as fertile or infertile according to their appearance (Omori, 1939). Female C. lectularius were provisionally scored as interspecifically mated if the ectospermalege seen through the abdominal wall of the live animal appeared very dark. The diagnosis was reappraised after the dissected ectospermalege had been immersed in lactophenol for at least twenty four hours, and examined under a microscope (Walpole, 1988).

Laboratory observations

Fifth stage nymphs of both species which had been caught in natural infestations were put into individual tubes. After adult emergence, the virgin insects were put together in a series of trials which were either of an experimental or correlational design. This work was carried out in a heated insectary where the mean temperature and relative humidity were 24,9 degrees C and 59,3%.

a) Experimental design research

In each experiment, virgin adults were randomly allocated to groups for comparison of the effects of inter- and intra-specific mating on longevity and egg production. Several experiments testing different combinations of inter- and intraspecific cohabitation were carried out, of which four are reported here in which virgin male C. hemipterus more than two weeks old were used. Each pair of bugs occupied a 50 x 14mm glass tube containing a rectangle of cardboard and topped with mosquito netting. Once a week, occasionally after two weeks, bugs were offered three opportunities to

feed on a shaved, sedated guinea pig through the mosquito netting and feeding performance was scored. Each week the cardboard rectangle and inside surfaces of each tube were checked for eggs which were removed and counted. Dead bugs were noted and removed each working day, deaths occurring over the weekend being scored as Saturday.

Bugs put together in cohabitation remained together until the death of the female C. lectularius, unless a shorter time was specified, when the male bug was removed. Analyses were carried out on the original data set using the Kruskal-Wallis (Siegel, 1956) and Dunn (Dunn, 1964) non-parametric tests. The former procedure is the equivalent of a one-way analysis of variance, whilst the latter effects pairwise comparisons.

b) Correlational design research.

Virgin bugs were put together in equal numbers into 57 x 35mm net-topped plastic tubes containing cardboard rectangles, according to the combination of sexes and species desired. Replicates of each experiment were started whenever sufficient material was available. Insects were thus not randomly allocated at the same time to the various groups, and the research design is correlational (Table 1). Bugs were fed as described in the above section, with mortality scored daily and eggs counted and removed weekly. The longevity of each species and sex in trials involving single species, both sexes of both species, and opposite sex

of different species observations, were compared using the Generalized Wilcoxon (Breslow) and Generalized Savage (Mantel-Cox) non-parametric tests (Breslow, 1974). The former test is most sensitive to differences in the earlier phase of life distribution data, whilst the latter puts more emphasis on the tail end. BMDP Statistical Software (University of California press, 1985 edition) was used.

Results

Data from the seven huts infested by both species (Table 2) showed that female C. lectularius are progressively more likely to be mated by C. hemipterus males the more the latter species predominates in the adult population. ($b = -0,92$; $t = 6,79$ for 5 degrees of freedom; $P < 0,002$).

Female C. lectularius are far more likely to lay only sterile eggs when they are captured from huts where C. lectularius constitute less than 28% of the adult bedbug population (Table 3) than when C. lectularius constitutes 72 to 97% (Fisher's Exact Test :- $P<0,001$).

Of the various types of collections made from huts, small or large collections of one or both species, containing interspecifically mated or not interspecifically mated C. lectularius females, only the interspecifically mated C. lectularius were prone to lay only sterile eggs (Table 4). This tendency was significantly more marked in interspecifically mated females than any other group (Fisher's Exact Test :- $P<0,05$).

Table 5 shows that female C. lectularius live longer cohabiting with males of their own species than with C. hemipterus males, and one mating with C. hemipterus does not reduce lifespan as much as cohabitation. One week of cohabitation with a C. hemipterus male can reduce the lifespan and production of fertile eggs, per day or per blood meal, and if a virgin female C. lectularius spends a week with a male of each species, its egg production per blood meal is lower if the C. hemipterus male cohabits first. Only female C. lectularius which had been observed mating with C. hemipterus males or showed the darkened ectospermalege were included in results pertaining to interspecific mating.

Figures 1 to 4 show respectively the survival of male C. lectularius, male C. hemipterus, female C. lectularius and female C. hemipterus when with equal numbers of the opposite sex of its own species; both sexes of both species; and the opposite sex of the other species. The discovery by Walpole (1988) of a marker for interspecific mating in C. lectularius females was made known to me when part of this work was still in progress, and all of 13 C. lectularius females cohabiting with C. hemipterus males, and 9 of 12 cohabiting with both sexes of both species were demonstrated to have mated interspecifically. The significance of differences in longevity are analysed in Table 6.

Table 7 compares fertile egg production when bedbug species are on their own or mixed. Laying of fertile eggs by C. lectularius females was greatly reduced when cohabiting with

C. hemipterus (chi-squared = 51,61; P<0,001), though the performance of C. hemipterus was better when the two species lived together (chi-squared = 7,97; P<0,01).

Discussion

The experimental data presented here (Table 5) confirm Omori's (1939) findings that the interspecific mating with C. hemipterus males does tend to reduce longevity and fertile egg production of C. lectularius females. Great variation between individual bugs is evident both in the present work and that of Omori (1939), in that some C. lectularius females died within a few days of interspecific mating or were rendered completely sterile, whereas others lived for months and laid several fertile eggs. As regards longevity, it is probable that the volume of sperm injected during interspecific mating, the number and temporal separation of such matings that the female C. lectularius experiences, and the female's individual degree of sensitivity to the alien sperm are all relevant.

The timing of interspecific mating, whether it occurs before or after the female C. lectularius has mated intra-specifically, is important to fertile egg production. Situations where C. hemipterus are the first to mate virgin C. lectularius females will be the most damaging to a C. lectularius population (Table 5), and this is most likely to happen in infestations where C. hemipterus predominates.

The correlational data (Figs 1 to 4; Table 6) show that

while the longevity of male C. hemipterus was unaffected by the sex or species of its cohabitants, its presence was associated with shorter survival times of other insects in the same container. This trend did not achieve significance in the case of C. lectularius males, though it is visible in Fig. 1. Female C. lectularius were the most affected in observations where C. hemipterus males were present, with 100 mortality occurring at 70 days in mixed species trials and only one female out of 23 survived more than 60 days cohabiting with C. hemipterus males (Fig. 2).

In the mixed-species trials, most of the female C. lectularius were interspecifically mated even though several conspecific mates were available for the C. hemipterus males (Table 1). While causation cannot strictly be inferred from correlational data, the elimination of the female C. lectularius group in all the mixed-species replicates accords with Omori's (1939) hypothesis. If male C. hemipterus are more sexually active than male C. lectularius, as suggested by Omori (1941), the debilitating effects of frequent mating may account for the better survival of female C. hemipterus in their absence. If male C. lectularius were affected, it could have been a result of homosexual activity (Rao, 1972).

The depression of the fertile egg production of C. lectularius when C. hemipterus is present (Table 7) supports the concept that interspecific mating is taking place to an extent that would threaten the continued survival of a C. lectularius population.

The field data (Table 2) showed interspecific mating occurred in all seven huts, with all female C. lectularius interspecifically mated when this species constituted only 5% of the total infestation and only 7% so mated when 96% of adult bugs caught were C. lectularius. Adverse effects of interspecific mating would therefore not seem to be an important factor regulating a C. lectularius infestation when a few C. hemipterus are also present. This view is supported by the fact that only 2% of C. lectularius females laid only sterile eggs when infestations contained over 75% C. lectularius, compared to the 58% laying three or more sterile eggs, and no fertile eggs, when C. hemipterus constituted over 70% of the bedbug infestation (Table 3). Virgin females never lay more than two sterile eggs (Davis, 1964), but mated females which have used up their sperm supply may lay batches of sterile eggs. It could be argued that this may happen in very small infestations if mating opportunities seldom occurred.

However, collections from lightly and heavily infested huts (Table 4) showed that only interspecifically mated C. lectularius females exhibited this behaviour to any extent.

The laboratory and field data presented above firmly indicate that interspecific mating does occur between C. hemipterus males and C. lectularius females in human domiciles in KwaZulu. This interaction severely prejudices the chances of survival of a small number of C. lectularius in a large C. hemipterus infestation, though when C. lectularius constitutes over 75% of the population, the

effects are considerably less serious or negligible.

It would therefore appear that a resident C. hemipterus population would prevent colonisation by C. lectularius if a few individuals were brought in from time to time. However, very large number of C. lectularius arriving in infested furniture, for example, might survive if the C. hemipterus infestation was greatly outnumbered.

Similarly, small numbers of C. hemipterus carried into a house heavily infested with C. lectularius will not affect the survival of the latter species, though a very large influx might.

Interspecific mating clearly plays an important role in deciding the abundance and distribution of C. lectularius in areas where both species are otherwise able to survive.

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TABLE 1. Correlational design research into survival and egg production when species are alone or mixed.

Observation	No. of insects in each replicate	Total
Single species, ♂ & ♀		
C. lectularius	8,20,20,16,20,10	94
Single species, ♂ & ♀		
C. hemipterus	6,10,20,16,14,10,10	86
Mixed species both sexes	8,16,12,20,20,24,16, 24,8,16	164
C. lectularius ♂ & C. hemipterus ♀	6,12,12,8,8	46
C. hemipterus ♂ & C. lectularius ♀	8,8,10,10,10	46

TABLE 2. The incidence of interspecifically mated female C. lectularius in seven huts harbouring both C. lectularius and C. hemipterus.

Hut	% C. lectularius in adult stage in mixed species infestation(N)	% C. lectularius which had been inter-specifically mated(N)
*1	5 (78)	100 (2)
2	19 (179)	72 (18)
*3	28 (97)	50 (12)
4	64 (25)	50 (8)
5	72 (93)	24 (34)
6	93 (64)	3 (34)
7	96 (272)	7 (111)

* Data included in Walpole & Newberry (1988).

TABLE 3. Percentage of female C. lectularius laying sterile eggs only after collection from huts containing C. lectularius and C. hemipterus

No. of Huts	No. of adult bedbugs of both species	% lectularius among adults	% laying lectularius++ which only produced sterile eggs in excess of two (N)
3	354	5 - 28	58,3 (12)
3	431	72 - 97	2,0 (149)
5	261	100	0 (136)

TABLE 4. Egg laying performance of female bedbugs from apparently small infestations (< 10 female bugs collected during a search) and large infestations (10 > female bugs collected per search) from huts containing single and mixed species. U - female not mated interspecifically. M - female mated interspecifically.

Species	Type of infestation in huts	Small or large infestation	No. of collections	No. of females laying fertile eggs	No. of females laying sterile eggs only (in excess of two)
C. lectularius	single species	small	6	16	0
C. lectularius(U)	mixed species	small	9	11	1
C. lectularius(M)	mixed species	small	10	7	9
C. lectularius(U)	mixed species	large	7	126	0
C. lectularius	single species	large	1	87	0
C. hemipterus	single species	small	5	12	0
C. hemipterus	mixed species	small	7	24	1
C. hemipterus	mixed species	large	2	26	1
C. hemipterus	single species	large	3	62	1

TABLE 5 Experiments comparing longevity and egg production of female C. lectularius kept in individual tubes under different mating regimes. Kruskal-Wallis-(KW) and Dunn- (D) test statistics used.

Comparison of experimental mating regimes	No.of replicates	Test statistics and significance longevity	fertile eggs per day	fertile eggs per blood meal
L^{φ} cohabit $H\sigma^{\prime}$ x L^{φ} cohabit $L\sigma^{\prime}$	6 7	KW = 4,81 P<0,05	-	-
L^{φ} cohabit $H\sigma^{\prime}$ x L^{φ} one mating $H\sigma^{\prime}$	8 8	D = 9,94 P<0,05	- -	- -
L^{φ} cohabit $H\sigma^{\prime}$ one week then cohabit $L\sigma^{\prime}$ one week x L^{φ} cohabit $L\sigma^{\prime}$ one week	6 6	D = 11,25 P<0,05	D = 15,25 P<0,05	D = 12,75 P<0,05
L^{φ} cohabit $H\sigma^{\prime}$ one week then cohabit $L\sigma^{\prime}$ one week x L^{φ} cohabit $L\sigma^{\prime}$ one week then cohabit $H\sigma^{\prime}$ one week	6 6	D = 5,33 N.S.	D = 10,25 N.S.	D = 12,08 P<0,05

TABLE 6. Comparison of longevity of C. lectularius - (L), and C. hemipterus - (H) in tubes containing: single species - (1), both species - (2), or males of one species and females of the other - (3). Statistical tests applied were the Generalised Wilcoxon (Breslow) - (GW) and the Generalised Savage (Mantel-Cox) - (GS).

Comparison of survival	Test statistics	Significance	Interpretation
H♂ 1 x H♂ 2 x H♂ 3 (n=43) (n=40) (n=22)	GW = 0,392 GS = 0,198	N.S. N.S.	H♂ not affected by ♂ or ♀ of same or other species
H♀ 1 x H♀ 2 (n=43) (n=42)	GW = 1,065 GS = 1,730	N.S. N.S.	H♀ not affected by presence of L
H♀ 1 x H♀ 3 (n=43) (n=23)	GW = 7,671 GS = 9,408	P<0,01 P<0,01	H♀ adversely affected by presence of H♂
H♀ 2 x H♀ 3 (n=42) (n=23)	GW = 4,451 GW = 4,493	P<0,05 P<0,05	H♀ adversely affected by presence of H♂
L♂ 1 x L♂ 2 (n=46) (n=41)	GW = 10,781 GS = 6,390	P<0,001 P<0,05	L♂ adversely affected by presence of H

Table 6. Continued.

Comparison of survival	Test statistics	Significance	Interpretation
L♂ 1 x L♂ 3 (n=46) (n=23)	GW = 2,632 GS = 0,556	N.S. N.S.	L♂ not affected by presence of H♀
L♂ 2 x L♂ 3 (n=41) (n=23)	GW = 0,187 GS = 0,754	N.S. N.S.	L♂ not affected by presence of H♂
L♀ 1 x L♀ 2 (n=47) (n=40)	GW = 45,763 GS = 52,035	P<0,001 P<0,001	L♀ adversely affected by presence of H
L♀ 1 x L♀ 3 (n=47) (n=23)	GW = 30,954 GS = 28,357	P<0,001 P<0,001	L♀ adversely affected by H♂
L♀ 2 x L♀ 3 (n=40) (n=23)	GW = 1,684 GS = 1,020	N.S. N.S.	L♀ not adversely affected by presence of H♀

TABLE 7. Production of eggs per bug-week (summation of female bedbugs present each week over duration of trial) of C. lectularius and C. hemipterus females when cohabiting with males of own species or males and females of both species.

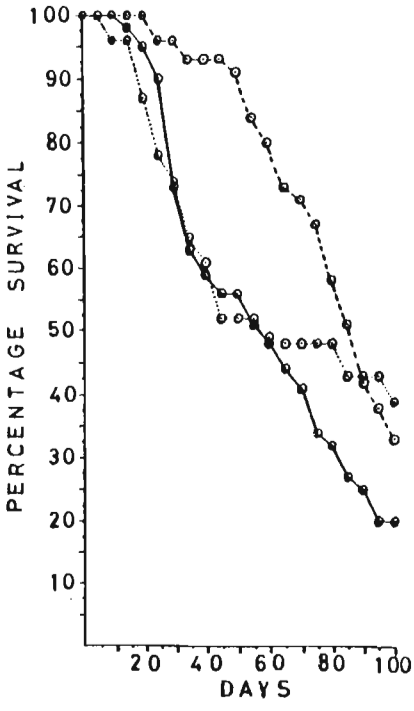
Species	cohabitation	No. of bug weeks	No. of fertile eggs	Eggs per bug week
C. lectularius	own species only	447	2636	5,90
	both species	160	448	2,80
C. hemipterus	own species only	299	678	2,27
	both species	293	873	2,98

FIG. 1. (Top left) Percentage survival up to 100 days of male C. lectularius when cohabiting with females of the same species (------); both sexes of both species (_____); and female C. hemipterus (.....).

FIG. 2. (Top right) Percentage survival up to 100 days of male C. hemipterus when cohabiting with females of the same species (------); both sexes of both species (_____); and female C. lectularius (.....).

FIG. 3. (Bottom left) Percentage survival up to 100 days of female C. lectularius when cohabiting with males of the same species (----------); both sexes of both species (_____); and male C. hemipterus (.....).

FIG. 4. (Bottom right) Percentage survival up to 100 days of female C. hemipterus when cohabiting with males of the same species (---------); both sexes of both species (_____); and male C. lectularius (.....).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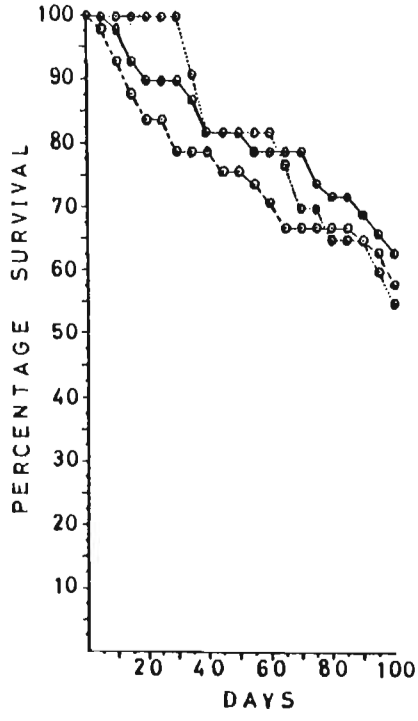
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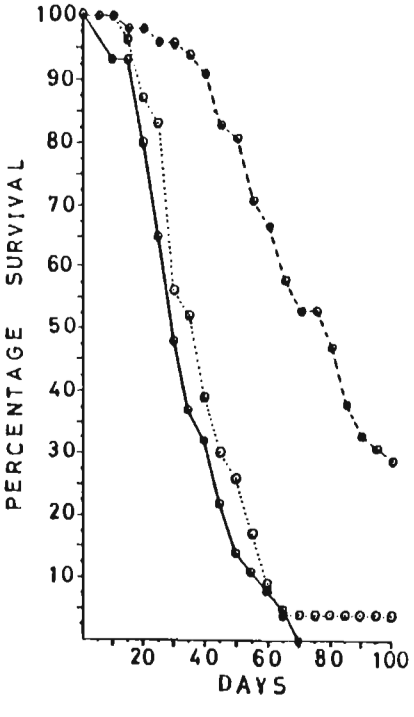
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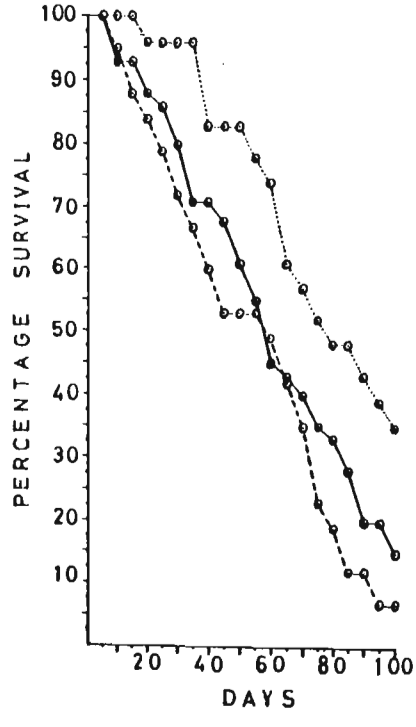
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Table 16. Longevity and egg production of female C. lectularius (L) under different mating regimes. "Young" C. hemipterus (H) males had fed only once after emergence, "old" males had fed two or more times. Some trials were terminated before the death of the insect (T)

Mating regime	female number	eggs laid fertile	eggs laid sterile	longevity (days)	full blood meals taken
1a) cohabit with old H male	1	0	0	15	1
	2	0	0	11	1
	3	0	0	45	2
	4	0	0	15	1
	5	0	0	5	1
	6	0	31	102 (T)	8
1b) cohabit with L male	1	44	2	102 (T)	6
	2	109	1	87	6
	3	159	3	102 (T)	9
	4	97	0	102 (T)	8
	5	75	1	102 (T)	8
	6	80	1	102 (T)	8
	7	0	0	15	1

Table 16. continued

Mating regime	female number	eggs laid fertile	eggs laid sterile	longevity (days)	full blood meals taken
2a) cohabit					
with old H male	1	0	0	7	1
	2	0	0	7	2
	3	0	6	14	2
	4	0	14	29	5
	5	0	0	4	1
	6	0	2	13	1
	7	0	1	149	8
	8	0	10	13	2
2b) one mating					
with old male H	1	0	6	74	7
	2	0	22	156 (T)	8
	3	0	2	66	6
	4	0	0	98	10
	5	0	1	66	5
	6	0	0	75	4
	7	0	9	13	2
	8	0	4	107	6

Table 16. continued

Mating regime	female number	eggs laid fertile	sterile	longevity (days)	full blood meals taken
3a) cohabit with L male	1	153	5	173	18
	2	275	1	219 (T)	22
	3	155	0	157	17
	4	112	3	181	17
3b) cohabit with young H male	1	0	2	22	2
	2	0	18	66	8
	3	0	11	97	10
	4	0	8	68	3
	5	0	17	147	13
	6	0	42	177	9
3c) 1 night with old H male then cohabit with L male	1	52	1	163	13
	2	0	0	4	1
	3	91	11	189	17
	4	0	0	4	1
	5	57	149	222	22
	6	67	33	222	21

Table 16. continued

Mating regime	female number	eggs laid fertile	eggs laid sterile	longevity (days)	full blood meals taken
3d) 1 night with L male	1	160	13	195	19
	2	332	8	222 (T)	23
	3	125	1	163	15
	4	136	0	177	14
	5	229	4	187	20
	6	203	2	212	20
4a) Cohabit with young H male for 1 week, then L male for 1 week	1	0	11	159	6
	2	33	39	159	8
	3	24	5	186 (T)	8
	4	0	0	7	1
	5	0	14	186 (T)	11
	6	16	18	122	9

Table 16. continued

Mating regime	female number	eggs fertile	laid sterile	longevity (days)	full blood meals taken
4b) Cohabit with old H male for 1 week, then L male for 1 week	1	0	0	7	1
	2	0	0	7	1
	3	0	1	7	1
	4	0	11	186	13
	5	11	12	117	6
	6	0	0	26	1
4c) Cohabit with L male for 1 week, then H male for 1 week (males used in 4b)	1	20	5	186	13
	2	15	1	32	1
	3	6	0	20	1
	4	70	2	129	6
	5	23	7	186	8
	6	67	1	147	6

Table 16. continued

Mating regime	female number	eggs fertile	laid sterile	longevity (days)	full blood meals taken
4d) Cohabit with					
L male for 1 week	1	82	16	136	11
	2	116	34	186 (T)	15
	3	102	5	186 (T)	16
	4	155	59	186 (T)	16
	5	71	10	186 (T)	17
	6	74	39	186 (T)	12

6.3 VARIATION IN THE LONGEVITY OF *Cimex lectularius* FEMALES MATED BY *Cimex hemipterus* MALES

Walpole (1988a) stated that the longevity of *C. lectularius* females up to 99 days was not reduced by a single mating with *C. hemipterus*. Her data are impossible to analyse in that two of her *C. lectularius* females were only observed for 47 days, four for 69 days, and the others between 73 and 99 days. Her controls, which were not randomly allocated and therefore form part of a correlational, not experimental, design, were also observed for differing lengths of time up to 128 days. The means of the longevities of conspecifically and interspecifically mated *C. lectularius* females given in her Table 10 (88 and 81 days respectively) are therefore meaningless (also erroneous in the latter case where sample size is given as 11 but is actually 12, resulting in a lower mean of 74 days). It should also be noted that although Omori (1939, 1941) and Walpole (1988a) give means for the longevities of their interspecifically mated *C. lectularius* females, the statistic is inappropriate. Interspecifically mated *C. lectularius* females tend to either have very short life spans of less than a month (Omori, 1939; present thesis, Table 16) or suffer no obvious reduction of longevity and survive for three months or more. Longevity therefore does not follow a normal distribution and non-parametric statistics and analyses are appropriate.

Walpole's (1988a) single interspecific matings did not result in the early death of C. lectularius females, whereas Omori's (1939) cohabitation trials often did. The different findings could be ascribed to the effects of single matings as opposed to presumed multiple matings except that in the present work (Table 16, 3b) three of six C. lectularius females each cohabiting with a newly emerged virgin C. hemipterus male lived for over three months, and two survived for over two months. This contrasts with the results recorded in Tables 1a and 2a, where only one of eight and two of six C. lectularius females survived one month's cohabitation with a twice-fed, two week old virgin C. hemipterus male. All the above results are explicable if one assumes that the death of C. lectularius females is caused by a large volume of C. hemipterus sperm injected either during one mating, or within a short interval of time by two or more matings. Sperm will build up in virgin males which are repeatedly fed but not allowed to mate, so C. lectularius females quickly died in trials (1a, 2a and 4b of Table 16) where such males were involved. Virgin male C. hemipterus which had only one feed did not usually inject a lethal dose of sperm (Table 16 3b, 4a) nor did older males which had previously mated (Table 16 4c). Where more than one C. hemipterus male is present, given a high frequency of matings in the laboratory (Cragg, 1923), female C. lectularius are likely to be interspecifically mated by two males in quick succession and thus receive a fatal dose. Statistical analyses are not appropriate for the results

considered in this paragraph since the data compared were carried out in different experiments.

6.4 INTERSPECIFIC FERTILITY : BETWEEN *Hesperocimex* SPECIES AND BETWEEN THE BEDBUGS

Ryckman & Ueshima (1964) found that though many female *Hesperocimex sonorensis* died after mating with *H. cochimiensis* (as described in 6.1.1 above), some crosses produced a low number of fertile eggs and a fertile F1 resulted. In the reciprocal cross, a high percentage of fertile eggs were laid and no female mortality was noted. Ryckman & Ueshima (1964) emphasised that as only some pairings produced fertile eggs, interspecific compatibility varied at the individual level. The same might also be said about female deaths. The direction of the cross was again important in another interspecific mating; 10 male *H. cochimiensis* fertilized 10 female *H. coloradensis* to produce 329 eggs of which only 14 were fertile and only two hatched, the parental females dying as did the F1 female after being back-crossed to *C. cochimiensis*. The reciprocal cross involving the same numbers of insects produced 906 eggs of which 834 were fertile and 813 hatched, though the F1 was sterile. The fate of the parental females is not mentioned. The three *Hesperocimex* species studied by Ryckman & Ueshima (1964) thus show both a strong incompatibility of fertilization systems often leading to heavy gametic mortality and the death of the female, and sufficient

compatibility such that in all six types of interspecific cross at least some eggs developed to the embryo stage, if not beyond.

A similar picture emerges when crossing the two South African bedbug species. Sometimes no eggs are laid, but other individuals may produce either a few or many sterile eggs (Table 16). Eggs with a normal appearance, as opposed to the shrunken form of sterile eggs (see Chapter Four) very occasionally result from the female C. lectularius and male C. hemipterus cross, and a hybrid has been produced by the reciprocal cross (Newberry, 1988). This latter paper illustrated by Plate 8 (page 139) is presented on pages 136 to 139 of this thesis and should be read at this point.

6.5 CONCLUSION

A degree of interspecific fertility (between C. lectularius and C. columbarius (Ueshima, 1964; Johnson, 1939); Hesperocimex species (Ryckman & Ueshima, 1964); C. lectularius and C. hemipterus (Newberry, 1988) is common in the Cimicidae, as is homosexual activity (Davis, 1966; Rao, 1972), interspecific mating in the laboratory (Ryckman & Ueshima, 1964; Ueshima, 1964; Omori, 1939) and toxic effects of interspecific mating to females often resulting in death (Omori, 1939; Ryckman & Ueshima, 1964; Newberry,(in press)). Though interspecific mating under natural conditions has only been demonstrated so far in the case of the two bedbugs

(Walpole & Newberry, 1988; Newberry, in press), it seems highly probable that this phenomenon is, like the above-mentioned behaviours, common among the Cimicidae. The curtailment of the distribution or abundance of one cimicid by a congeneric species brought about by the toxic effects of interspecific mating on the female may therefore not be a rare occurrence when cimicids are sympatric.

Medical and Veterinary Entomology (1988) **2**, 297–300

Production of a hybrid between the bedbugs *Cimex hemipterus* and *Cimex lectularius*

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ABSTRACT. Of 479 eggs laid by female *Cimex lectularius* Linnaeus and *C.hemipterus* (Fabricius) mated by males of the other species, one was fertile and gave rise to a first stage nymph. The egg showed sculpturing typical of *C.hemipterus*, the female parent, and the nymph conformed to the narrow pronotum and abdomen of this species, being significantly different from *C.lectularius* in the width of the abdomen. Because the orientation and lengths of the bristles on the sides of the abdomen were distinctly different from *C.hemipterus* and closely resembled *C.lectularius*, the single nymph obtained from the cross *C.hemipterus*×*lectularius* was interpreted as being a hybrid rather than a product of parthenogenesis.

Key words. *Cimex hemipterus, Cimex lectularius*, bedbugs, hybridization, hybrid nymph.

Introduction

It is frequently stated in the literature that only sterile eggs result from crossing *Cimex lectularius* Linnaeus and *C.hemipterus* (Fabricius), and that no hybrid has yet been produced (Mellanby, 1935; Omori, 1939; W.H.O., 1982).

Usinger (1966, p. 328) states, however, that 'Attempts by Hase, Omori, Davis and myself to cross *hemipterus* with *lectularius*, while occasionally producing eggs in the F1, failed to produce F2 adults'. None of those workers appear to have published an account of successful hybridization between the two bedbug species, so Usinger's (1966) statement is unsubstantiated.

This paper describes a first stage nymph which emerged from an egg laid by a female *C.hemipterus* mated solely with a male *C.lectularius*.

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Materials and Methods

Fifth-stage nymphs of both bedbug species were caught from human habitations in KwaZulu, where they live in sympatry (Newberry *et al.*, 1987; Walpole & Newberry, 1988). The nymphs were isolated until they developed into adults, when they were paired with an interspecific mate. Each pair of bugs resided in a net-topped glass tube containing a small rectangle of cardboard. Once a week, bugs were fed on a guinea-pig through the net tops of their tubes, and tubes and cardboard rectangles were checked for eggs, which were removed and allowed to incubate.

The hybrid first-stage nymph was preserved in 70% alcohol. For measurements the specimen

was placed on a slide and allowed to dry. First-stage nymphs emerged from eggs laid by wild-caught females were preserved and studied in the same way. Measurements of the pronotum and of the abdomen at its widest point were taken of thirty unfed stage 1 nymphs of each species, and of the hybrid. A micrometer eyepiece was used and the arbitrary units of measurement converted to millimetres. Photographs were taken using a scanning electron microscope at 42×magnification following standard preparation of the material.

Results

A total of 172 eggs were produced by twenty-five female *C.lectularius* mated with male *C.hemipterus*. All except three of these eggs had the usual shrunken, narrow appearance of a sterile egg. The three exceptional eggs resembled fertile eggs in size and appearance for several weeks, but no embryonic development could be seen and the eggs eventually collapsed.

Eight female *C.hemipterus* mated with male *C.lectularius* produced a total of 307 eggs, all except one being of the shrunken, sterile kind. One egg, laid along with nine sterile eggs, during the course of a week, had the appearance and sculpturing of a normal *C.hemipterus* egg. Red eye-spots were noted as the embryo matured and a first stage nymph was found, along with the egg shell, a few days later. The nymph was moribund and did not feed when placed on the author's hand.

Although their ranges of size overlap, *C.lectularius* first-stage nymphs are significantly broader across the pronotum and abdomen than *C.hemipterus* (t=4.3 and 12.1, respectively, for 58 degrees of freedom, $P<0.001$). From Table 1 it can be seen that the hybrid's measurements fall within the 95% confidence limits about the means of the two measurements for *C.hemipterus*, but lie clearly outside the 95% confidence limits about the mean of the abdominal measurements of *C.lectularius*, and on the lower confidence bracket of the mean of the pronotum widths.

The hairs along the lateral sides of the abdomen of *C.hemipterus* first-stage nymphs alternate between long bristles and shorter bristles a half or a third their length (Fig. 1a). Bristles in the same area of *C.lectularius* first-stage nymphs alternately vary by about 20% in length and are more backward pointing than those of *C.hemipterus* (Fig. 1c). The hybrid's lateral abdominal bristles resemble *C.lectularius* as regards hair length, and are even more pronounced in their backward grooming (Fig. 1b). Hairs on the body and legs of the hybrid tend to be more bent and less uniform in orientation than in the nymphs of the parent species.

Discussion

Virgin female *C.lectularius* can lay up to two sterile eggs (Davis, 1964), but sperm must be present in the oviducts or ovarioles for larger numbers of eggs to be produced (Davis, 1965). Female bedbugs which are interspecifically mated often lay large numbers of eggs. The fertilization systems of both species therefore must be sufficiently compatible to allow sperm from each other species to negotiate the mesospermalege, pass across the body cavity, penetrate the seminal conceptacles, rise up the oviducts and stimulate the corpus allatum to produce eggs. The production of a hybrid proves that successful completion of even later stages of post-mating specific-mate recognition (Paterson, 1985) and the development of a zygote are possible, though one of these phases is usually incompetent.

TABLE 1. Width (millimetres) of pronotum and abdomen, at widest point, of unfed first stage nymphs of *Cimex* spp.

Measurement	Species	No. in sample	Mean	95% confidence limits
Abdomen	*C.lectularius*	30	0.75	0.69–0.81
	C.hemipterus	30	0.66	0.61–0.71
	Hybrid	1	0.64	
Pronotum	*C.lectularius*	30	0.47	0.43–0.50
	C.hemipterus	30	0.44	0.41–0.48
	Hybrid	1	0.43	

Lewis (1949, p: 298) reported that 'In one or two individuals, amongst thousands examined, the shape of the prothorax was intermediate between *Cimex hemipterus* and *C.lectularius*'. However, hybrids may not be intermediate in this character and could therefore escape detection.

Since eggs from cross-mated females of *C.lectularius* and *C.hemipterus* are usually sterile, it is worth considering whether the single 'hybrid' nymph may have resulted from some form of parthenogenesis such as gynogenesis, i.e. spermatozoal stimulation of the ovum leading to embryogenesis. Parthenogenesis sometimes gives rise to female *Culex pipiens* L. *s.l.* (Laven, 1956; Kitzmiller, 1959) and *Aedes aegypti* (L.) (Craig, 1957) among mosquitoes, for example. However, the intermediate chaetotaxy of the nymph is taken as evidence of its true hybrid status.

The two bedbug species have been found together in 10% of infested huts in KwaZulu, South Africa (Newberry *et al.*, 1987) where human dwellings often support thousands of bugs (Newberry & Jansen, 1986) and interspecific mating has been found to occur (Walpole & Newberry, 1988). Hybrids may therefore be produced in the wild, and the possibility exists that fertile hybrids occur which allow gene flow between these two species of human ectoparasites.

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Plate 8. First stage nymphs of C. lectularius, C. hemipterus and a F1 hybrid of a female C. hemipterus x male C. lectularius cross. The hybrid's bristles appear degenerate in comparison to both the parent species, though resemble C. lectularius in their backward slant and the greater uniformity of length of the posterior lateral abdominal bristles.

Cimex lectularius

first stage nymph

0,43 mm

hybrid

first stage nymph

0,43 mm

Cimex hemipterus

first stage nymph

0,38 mm

CHAPTER SEVEN

THE TAXONOMIC STATUS OF *Cimex lectularius* AND *Cimex hemipterus*

7.1 INTRODUCTION

The delineation of the species *C. lectularius* has been questioned on two occasions. Johnson (1939) wished to bracket this anthropophilic bedbug with the pigeon bug *C. columbarius* as subspecies. Walpole (1988a) suggested that *C. lectularius* and *C. hemipterus* were two subspecies of the same Recognition species (Paterson, 1985).

7.2 *Cimex lectularius* AND *Cimex columbarius*

Mayr (1969) defined a subspecies as follows : "A subspecies is an aggregate of phenotypically similar populations of a species, inhabiting a geographic subdivision of the range of a species, and differing taxonomically from other such populations of the species." Mayr (1969) also said, "If two discrete breeding populations coexist at the same locality, they are full species (except in the rare case of "circular overlap.")"

Johnson (1939) allocated bugs to the taxa *C. lectularius* or *C. columbarius* on the basis of host association. He went on to show that although the two species were morphologically very similar they could usually be separated by their

different head-width : length of third antennal segment ratios (Chapter Two). He produced fertile hybrids from both reciprocal crosses, and on the basis of his findings Johnson (1939) decided that the two bedbug types should be regarded as subspecies of a single species.

Ueshima (1964) disputed this conclusion on the grounds that the two bedbugs infest the same houses in the Netherlands and are therefore sympatric, not being confined to their hosts as are many ectoparasites (Clay, 1949) and contact therefore being possible. Ueshima (1964) also stated that "reproductive isolation was found between" the two species in that a degree of positive assortative mating was evident in the laboratory, and one of the crosses (C. columbarius female and C. lectularius male) produced few eggs.

Ueshima (1964) drew conclusions that went beyond his data when he stated that the two bedbug types are definitely two species. The bugs may infest the same houses, but C. columbarius may dwell only in the roof with its avian hosts while C. lectularius remains in the rooms with the human occupants. The two insects may never meet, and thus be allopatric in the sense of Clay (1949).

Although a degree of positive assortative mating is apparent, between ten and 50% of C. lectularius females were mated by C. columbarius males even though equal numbers of C. columbarius females were present, and between ten and 30%

of C. columbarius females were mated by C. lectularius males under reciprocal conditions (Ueshima, 1964). If these two species intermate as readily in their natural habitat as they do in the laboratory, there would be a considerable production of hybrids which are fertile with themselves and at least one of the parent species (Johnson, 1939). The degree of reproductive "isolation" is therefore insufficient to prevent a merging of two sympatric infestations of the two bug types unless incompatible specific-mate recognition systems (Paterson, 1985) prevent cross mating in nature.

The degree of sympatry, and the amount of interspecific mating that actually occurs in natural habitats need to be known before any conclusion can be reached on the taxonomic status of these two bugs. Before this is possible, reliable methods of distinguishing the insects must be found.

7.3 Cimex lectularius AND C. hemipterus

The Biological Species concept essentially states that species are groups of interbreeding populations reproductively isolated from other such groups (Vrba, 1985). The production of fertile offspring is thus the key element in this species concept. The Recognition Concept defines a species as, "that most inclusive population of individual biparental organisms which share a common fetilization system" (Paterson, 1985). In the Recognition Concept, sterility is considered an intraspecific phenomenon if

fertilization is achieved, events after fertilization being considered irrelevant to the delineation of species.

Paterson (1984) warned that, "An author....can....glide imperceptibly from discussing species under one concept to talking about them under the other. This glide generates a subtle kind of nonsense...." This is very much what has happened to Walpole's (1988a) discussion of species delineation as regards C. lectularius and C. hemipterus, in addition to other serious flaws in her argument. Mayr's (1969) definition of a subspecies (quoted in 7.2 above) was coined in the context of the Biological Species concept, and he made it very clear that if two discrete populations coexist at the same locality, they are full species. This is precisely the situation with the two bedbugs, but presumably because they crossmate in nature (Walpole & Newberry, 1988), Walpole (1988a) wished to accord them subspecific status according to the Recognition Concept, and she attempted to argue this point in terms of Mayr's (1969) definition of a subspecies.

If the two bedbug types were totally allopatric, and their intersterility of no importance, there would be a case for regarding them as subspecies. Walpole (1988a) tried to approach this position by saying the two bedbugs are rarely in sympatry (which is unlikely to be true, given that they coexist in many tropical countries (Usinger, 1966) and can flourish in the same geographical areas, as in KwaZulu

(Chapter Three.)) She also implied that a little sympatry may be waived as allowable "flexibility," though it is certain that Mayr (1969) did not intend the category of subspecies to include intersterile populations. Walpole (1988a) tackled the problem of intersterility by substituting the Recognition Concept species for the Biological species in Mayr's (1969) discussion, since in the former concept sterility is regarded as irrelevant to species delineation. As the two species concepts differ radically on the importance of sterility between populations, Walpole's conflation (merging of two distinct concepts (Paterson, 1984)) of species definitions destroys the logic of an already factually uncertain argument.

Walpole's conflation of the Recognition Concept of species with the Biological species leads to further confusion when, in her section on the former concept, she states that the two bedbug types are not morphs of a single population. Ford's (1940) definition of polymorphism is: "The occurrence in the same locality of two or more discontinuous forms of species in such proportions that the rarest of them cannot be maintained by recurrent mutation." In fact, if one substitutes the Recognition Concept species into this definition, as Walpole (1988a) appears to be doing, a polymorphism is exactly what we have.

It is obvious from the above that persons wishing to use terms such as subspecies and polymorphism in the context of

the Recognition Concept of species must coin new definitions for these terms.

One of the tenets of the Recognition Concept of species is that natural selection for reproductive isolation between two populations very rarely, if ever, occurs. Paterson (1978) presented a model which showed that in the case of two randomly mating, intersterile populations of the same species, the less numerous of the two would rapidly go to extinction. The reason for this is that ova are used up in sterile unions with incompatible sperm when interpopulation mating takes place. With random mating, most sexual encounters will be of the sterile kind in the case of the rarer population, and the situation will worsen each generation until it is eliminated.

Walpole (1988a) used Paterson's (1978) model to explain the absence of C. lectularius from the Orient, where C. hemipterus is the more abundant species (Omori, 1939). However, a consideration of the consequences of interspecific mating between the bedbugs shows that Paterson's (1978) model is entirely irrelevant. Interspecific mating does not greatly affect the fertility of C. hemipterus females, if at all (Omori, 1939; Chapter Six), presumably because syngamy is rarely achieved. The fertile egg production of C. lectularius females is unlikely to be reduced because of a wastage of ova by fusion with C. hemipterus sperm, because the alien sperm provoke an immune

response in the female and are subjected to melanotic cellular encapsulation (Walpole, 1988b; Gotz & Bowman, 1985). This immune reaction, and the toxic effect of C. hemipterus seminal fluid on female C. lectularius tend to reduce the lifespan and fertility of the latter, but this has nothing to do with Paterson's (1978) model. The latter model predicts that either species can exterminate the other and the more numerous species will be the survivor. However, the deleterious effects of interspecific mating between the bedbug species are unidirectional, with C. lectularius populations being replaced by C. hemipterus even when the latter species is initially the less numerous (Newberry et al, 1987).

Walpole repeated this conceptual error when she mentioned the findings of Newberry et al. (1987) as a further example of Paterson's (1978) model, and added to the confusion by misinterpreting the data she referred to. Newberry et al. (1987) reported that in Zulu huts which initially harboured both species of bedbug, C. hemipterus tended to displace C. lectularius over a period of months. Walpole (1988a) stated that, "Displacement of C lectularius by C. hemipterus, where C. hemipterus is the larger of the two populations, appears to be occurring in some huts..... . Where C. lectularius is more abundant, C. hemipterus is either present in low numbers or not at all (Newberry et al., 1987)." Walpole's account is incomplete in that C. lectularius was replaced or outnumbered by C. hemipterus in huts where the latter was

not initially the more numerous (huts A, C and D, Table 1, Newberry et al., 1987, page 54 of this thesis). Also, the continued existence of small numbers of C. hemipterus in huts where C. lectularius was the more abundant species demonstrates that the former species is not eliminated by the latter even when greatly outnumbered (huts F and G, Table 1, Newberry et al., 1987).

Although there are no grounds whatever for Walpole's (1988a) assertion that C. lectularius and C. hemipterus should be regarded as subspecies, their taxonomic status under the Recognition Concept of species is in need of clarification. This problem was explored in a paper by Newberry & Brothers (in press) reproduced on pages 148 to 160 of this thesis, and should be read at this point.

In conclusion, it seems clear that the Recognition Concept of species does lose contact with its theoretical grounding by subscribing to the concept of a species as a field for gene recombination whilst at the same time denying the importance of sterility as a barrier to gene flow (Paterson, 1985). The Biological Species concept does not suffer from this major inconsistency and is therefore preferable. Under the latter species concept, the two bedbugs are unequivocally separate species.

Problems in the Recognition Concept of species: an example from the field

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Theoretical and practical problems in the application of the Recognition Concept (RC) of species to the anthropophilic bedbugs, Cimex lectularius Linnaeus and C. hemipterus (Fabricius), are discussed. These extensively sympatric bugs undergo considerable cross-mating in their natural habitat in KwaZulu, South Africa, although the production of hybrids is extremely rare. It is concluded that the RC is inadequate for species delimitation under such circumstances.

Paterson (1) defines a species as `that most inclusive population of individual biparental organisms which share a common fertilization system', including some means of specific-mate recognition, in its normal habitat. This Recognition Concept (RC) of species attempts to put Carson's (2) theoretical definition, that the species is a field for

gene recombination, into an operational form superior to (3) (and thus more readily testable than) the Biological Species Concept (BSC), which essentially maintains that species are groups of interbreeding populations reproductively isolated from other such groups.(4) The RC is also purported to be nonrelational and thus more directly applicable to actual species-as-individuals than is the relational BSC, although the validity of this claim is highly questionable. (5, 6) The RC considers reproductive isolation in the sense of interpopulational sterility irrelevant to the delimitation of species, (1) and is thus not obliged, as is the BSC, to recognize cytoplasmically incompatible strains (7) as separate species. However, two intersterile populations cannot be part of the same field for gene recombination, so contact between the operational RC definition and its theoretical grounding appears here to have been lost. (5, 6) Paterson solves this problem algebraically by predicting that in the case of two randomly-mating, intersterile populations which share a common fertilization system, are equally fit and have the same ecological requirements in complete sympatry, the less numerous population or chromosome arrangement will swiftly die out. (8) He thus portrays such intersterility as a transient intraspecific phenomenon. This may very well be true under that set of rather rigid assumptions, but the result may be very different if not all apply (which is highly likely in nature), in which case a particular Recognition species may not correspond to a single field of gene recombination. If

only fitness differs and the less fit population is the smaller it will certainly be eliminated quickly, but if it is the larger it may still be eliminated, although over a longer period, if the difference in fitness is great enough. If only the ecological requirements differ, the microdistribution may consequently differ such that mating occurs less frequently between individuals from different populations, in which case both could persist for long periods. And if only the distributions differ such that the populations are widespread and only partly sympatric, both may also persist (9) in parapatry or allopatry. Any combination of differences in more than one characteristic will exacerbate these effects. In such circumstances the RC is patently inadequate for delimitation of significant genetic units, despite Paterson's (1) statement (p. 25) that `the limits to a `field for gene recombination' can be set by the fertilization system of members of a population of organisms alone.' Instead, the intersterility itself must be a very real factor affecting the limits of the gene pools. If the RC were to be expanded to take this into consideration, it would become explicitly relational and virtually indistinguishable from the BSC.

Consideration of two anthropophilic bedbugs may be instructive in this context. Cimex lectularius Linneaus has virtually world-wide distribution though it is absent from some areas of the Orient , and C. hemipterus (Fabricius) occurs throughout the tropics, or close to them.(10) Although both feed predominantly on human hosts, they are

morphologically and physiologically distinct and reproductively isolated, and are thus considered to be good Biological species. They appear to have very similar although not identical ecological requirements, and occur sympatrically in northern Natal and KwaZulu, where 10% of infested human habitations were found to harbour both. (11) Mating between C. lectularius and C. hemipterus has been observed in the laboratory (10) and elsewhere. (12) The vast majority of eggs laid by females only cross-mated are sterile, but hybrids of limited viability have very infrequently been produced. (10,13) Females of C. hemipterus mated by males of both types are apparently minimally affected, laying an approximately normal complement of fertile (nonhybrid) eggs and having a normal life span; by contrast, females of C. lectularius mated by males of both types generally lay fewer fertile eggs than normal or none at all and very often have a shortened life span. (14) Females of C. lectularius mated by males of C. hemipterus develop a black mark in the extospermalege one to seven days after mating. (15) The mark is apparently a melanotic cellular encapsulation (16) brought about by the insect's immune system acting on the foreign sperm. This mark was seen in over two-thirds (n = 16) of the females of C. lectularius in two double-infested huts in KwaZulu, (12) proving that interspecific mating is common. Collections from five more huts have shown that the percentage of interspecifically mated female C. lectularius increases with increasing rarity of adult C. lectularius in double-infested

huts ($b = -0,92$; $t = 6,79$; $P < 0,002$), ranging from 100% where C. lectularius formed only 5% of the adult population, to 7% where they comprised 96% of the adult bedbugs.(17) The common occurrence of interspecific mating is further indicated by the high percentage (58,3%) of sterile eggs laid by C. lectularius females in huts where C. lectularius adults constituted 5-28% of the population.(17) Males of C. lectularius inseminate females of C. hemipterus in the laboratory, but no marker of this event has been discovered to enable verification that such cross-matings occur in the field.

The status of these two types of bedbug in terms of the RC rests on evaluation of the extent to which they share a common fertilization system in their normal habitat. Paterson(1) is unfortunately not clear as to the true extent of the fertilization system, even if one limits consideration to animals. His statement (p. 24) that `each fertilization system comprises a number of components... which contributes to the ultimate function of bringing about fertilzation´ agrees with his formal definition of a Recognition species. He further states (p. 27) that `all phenomena covered by the category `postmating isolating mechanisms´ (Mayr, 1963) ... have nothing to do with bringing about fertilization.´ The first such postmating isolating mechanism listed by Mayr (18) is gametic mortality where sperm transfer takes place but syngamy does not occur because of antigenic reactions in the genital tract of the female or for other reasons. Here Paterson clearly

differentiates between fertilization and syngamy, and the 'fertilization system' in animals thus appears to be limited to those processes which lead to successful copulation. A part of the fertilization system is the Specific-Mate Recognition System (SMRS),(1) which involves a set of reciprocal signals and responses whereby appropriate mates are brought together. Paterson states that the SMRS is the major part of the fertilization system in mobile organisms but 'in sessile animals it may be restricted to the recognition of the sperm by the ovum' (p. 25), in which case the distinction between fertilization and syngamy seems to have disappeared.

If the fertilization system is indeed limited to the processes leading to successful mating, then there is good evidence that C. lectularius and C. hemipterus share a common fertilization system since cross-matings are apparently frequent in nature, and they should thus be considered to belong to the same Recognition species. On the other hand, if the fertilization system includes all processes up to syngamy (a concept which is perhaps more easily generalized across all sexually reproducing organisms), then there are probably differences in those components of their fertilization systems which operate after copulation such that syngamy does not usually occur after cross-mating, and they may thus be different Recognition species after all. (The actual point at which such breakdown occurs is not known, but is perhaps more likely to involve gametic mortality than zygote mortality

since more than 99% of eggs laid by cross-mated females were shrunken and obviously sterile at laying, and most of the normal-appearing eggs did not develop.)(13))

How is one to judge whether fertilization systems are shared (held in common) or not? If the system does not extend beyond successful copulation the decision may be relatively simple in motile animals (although the degree of `success´ may actually be difficult to evaluate), but if it is considered to operate up to syngamy then it is far less easy, since direct evidence on the actual occurence or not of syngamy is needed. Evidence for syngamy, of course, includes the production of any offspring after cross-mating. If any occurrence of syngamy between individuals of two populations in nature, however rare, indicates a common fertilization system and thus conspecificity, then hybridization between Recognition species is a logical impossibility. However, Paterson´s statement that hybridization merely `involves a process having something in common with a cross-reaction´ (1) when the system of specific responses to specific signals is analogized with a series of antigen-antibody reactions (p. 25), shows that he accepts the possibility of hybridization. The frequency of successful achievement of syngamy must thus be important in establishing the degree of commonality of fertilization systems, and thus in delimiting Recognition species. In that case a grey area exists, similar to that in the BSC where arbitrary decisions have to be made concerning the degree of interfertility that can be regarded as acceptable between

Biological species.(6) Should a situation where an occasional `hybrid´ individual is produced as a result of interactions between two populations in which cross-mating is frequent, be interpreted as one in which the fertilization systems are different (but there is the odd insignificant error) and more than one species is involved, or as one in which the fertilization systems are the same (but there is some factor, irrelevant to the specific status of the populations, which usually causes intersterility) and only one species is involved? This is the situation which probably appertains in the case of the anthropophilic budbugs, so that their status in terms of the RC remains equivocal.

The RC further requires that the fertilization system be considered when operating in the `preferred environment´(19) or `normal habitat´(1) of the organisms involved. Decisions on this are also problematic. Bedbugs living in a hut in KwaZulu today are subject to several unnatural environmental disturbances such as cooking fires, replastering of mud walls and annual spraying with DDT (to which they have developed a high degree of resistance). They thrive there in amazing numbers, (20) and this must be considered to be their `normal habitat´ in that region. Conditions in such a hut are undoubtedly different from those pertaining when and where the two bedbug types arose, however, and it is thus possible that there is interference with the subtle balance of components in a SMRS which was evolved under other circumstances. In order to minimize such problems, it is

probably more appropriate to investigate populations in their primordial habitats. In the case of the anthropophilic bedbugs, we cannot know the exact nature of that habitat. Does this mean that we can therefore make no decision on the specific status of the bedbugs? A requirement that organisms can only be assessed in their original habitat in terms of the RC is idealistic and impractical.

From the above, it can be seen that the RC is by no means always a problem-free superior alternative to the BSC. In fact, both concepts suffer from difficulties of application under various conditions. This is a common failing of operational definitions which have to be modified when circumstances are discovered where they cannot be used. Such definitions may actually prove to be special cases within less restrictive and more heuristic(21) theoretical definitions,(22) such as the Evolutionary Species Concept (22,23) which considers all available criteria (including those utilized by the BSC and the RC, as well as others) in order to estimate the limits of particular species. Such a theoretical definition is likely to prove of greater general applicability and thus of more lasting worth in the extremely varied world in which organisms operate. This does not mean that the insights provided by the RC and the BSC should be ignored, bit rather that they should be relied upon only in those circumstances where conditions are entirely appropriate for their application. In the present case, that of the bedbugs, the perspective provided by the BSC seems to be more useful than that of the RC.

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CHAPTER EIGHT

THE INSECTICIDAL CONTROL OF BEDBUGS

8.1 INTRODUCTION

Before the Second World War, a variety of methods were used to combat bedbug infestation which had become a serious problem in cities (Usinger, 1966). General hygiene proved to be of limited use because bedbugs often hide in inaccessible places e.g. cracks in walls. Attempts were sometimes made to raise the temperature of rooms above 45 degrees C which exceeds the thermal death point for all life stages of both C. lectularius and C. hemipterus if maintained for over one hour (Mellanby, 1935). Many "infallible" mixtures were available for spraying against bedbugs which may have had some flushing effect but no residual life or toxic action stronger than that of soap and water (Young, 1938).

Fumigation using sulphur dioxide, ethylene oxide or hydrogen cyanide could give satisfactory results (Usinger, 1966). The latter two chemicals were dangerous and inconvenient to use, however, with neighbouring houses having to be vacated and great care taken to ensure that no gas remained in a house before reoccupation (Young, 1938). Chlorinated hydrocarbon residual insecticides became available during World War II and proved to be extremely effective for bedbug control all over the world. However, as early as 1947 DDT resistance was reported from Hawaii (Johnson and Hill, 1948), and incidents

of bedbug resistance to DDT, dieldrin and benzene hexachloride (BHC) began to occur in several countries (Busvine, 1958). Over the years, organochlorine resistance spread until it was very common in C. hemipterus and almost universal in C. lectularius (World Health Organisation (WHO), 1980), and appears to have become a problem in South Africa in the early 1960's (Whitehead, 1962). Spraying now seemed to encourage bedbug infestations rather than control them, and anti-malaria spraying teams encountered lack of cooperation from hut occupants involving locked huts and forbidden entry. Rafatjah (1971) stated that of 32 countries considered, 19 found bedbug infestations caused problems in their malaria control projects. Ewers (1972) reported that DDT resistant bedbugs were increasing in numbers in Papua-New Guinea, and attributed this to the destruction of predators such as lizards and spiders by the DDT. Bourke (1973) presented very preliminary data from the same country suggesting that bedbug infestation occurs more readily in houses treated with DDT.

Bedbugs may be resistant to DDT, methoxychlor and their analogues, or to dieldrin, BHC, and other chlorinated cyclodienes. Thus some C. hemipterus from Hong Kong were resistant to DDT but not to dieldrin, whilst the reverse applied to the same species from Tanganyika (Tanzania) (Busvine, 1958). However, Shalaby (1970) reported C. lectularius resistant to both DDT and dieldrin though only the former was ever used in the area of Libya concerned. In

the United Arab Republic, DDT and dieldrin resistance in C. lectularius have both been shown to be due to monofactorial inheritance having a semi-dominant expression (Gaaboub, 1971).

If the treatment of a house with insecticide does not lead to control, the reason may be inadequate coverage of bedbug harbourages or too low a dosage rate of the chemical. If these are sufficient, the problem may be the ability of individuals in the infestation to survive exposure to the insecticide used. Tests are then carried out using known concentrations of the insecticide to assess the susceptibility of the bedbugs.

Rao and Halgeri (1956) used DDT powder in various dilutions in talcum powder to measure the susceptibility of bedbugs in Bombay. Lofgren et al. (1958) used wool treated with DDT in acetone. Many studies, e.g. Busvine (1958) and Gaaboub (1971), have used impregnated papers issued by the WHO (WHO, 1975). The WHO papers presumably have the advantage of uniformity in preparation, though the use of a thick, rare, Swiss-made filter paper and mineral oil as solvent is not regarded an ideal for all insecticides (A J R Pope, pers.comm.*). Impregnated papers for insecticides other than the organochlorines can also have a very limited shelf life,

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and difficulty can be experienced obtaining them from WHO.

The WHO impregnated papers are prepared by dipping filter papers into various concentrations of insecticide, e.g., 4% DDT. It is not known how this measurement of concentration relates to sprayed insecticide in which dosage rates are expressed as grams per square metre (g m-2). Reid (1960), made his own malathion papers for testing bedbugs in Malaya, though he adopted percentage concentration rather than g m-2 to calibrate dosage. The technique of topical application using a micropipette was used by Feroz (1968) to study organophosphorus resistance in C. lectularius from Israel.

Following the spread of bedbug resistance to the organochlorines in many countries, a number of publications have appeared announcing the successful use of other insecticides chosen, one assumes, for their local cost and availability. Lofgren et al. (1958) found diazinon the fastest acting of the eleven effective insecticides they tested. Malathion has been used successfully in Malaya (Reid, 1960) and Israel (Rosen et al., 1987), but proved unsatisfactory in India (Shetty et al., 1975; Varma & DuttaGupta, 1983) where fenitrothion has proved to be superior. In South Africa, Whitehead (1962) achieved control of bedbugs resistant to DDT and dieldrin in a hostel by using carbaryl at 1 g m-2.

In the present work, four lines of research were pursued to:

1) Consider the relationship between the spraying of huts

with DDT and bedbug infestation, 2) Determine whether there were bedbug predators among spider species in mud huts, 3) Establish whether resistance to DDT and dieldrin existed in C. lectularius and C. hemipterus populations in KwaZulu, 4) Test candidate insecticides for their usefulness in bedbug control under field conditions. A presentation of these projects follows.

8.2 BEDBUG INFESTATION AND INTRADOMICILIARY SPRAYING OF RESIDUAL INSECTICIDE IN KWAZULU

This work was reported in Newberry et al. (1984) which is reproduced on page 166 and should be read at this point.

8.3 SPIDERS AS POSSIBLE PREDATORS OF BEDBUGS

The eradication of bedbug predators by spraying huts with DDT during malaria control operations has been assumed to be the cause of increases in bedbug numbers in New Guinea (Ewers, 1972) and was suggested as an explanation for widespread bedbug infestation in KwaZulu (Newberry et al., 1984). Povolny (cited by Usinger, 1966) reported that an infestation of C. lectularius feeding on bats at Austerlitz was destroyed by the spider Steaboda bipunctata. Lorando (cited by Usinger, 1966) asserted that another spider, Thanatos flavidus, eradicated all the budbugs in refugee camps in Greece in the 1920's, and this spider also proved effective when tested in the laboratory (Hase, cited by

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Page 377.

Bedbug Infestation and Intradomiciliary Spraying of Residual Insecticide in KwaZulu, South Africa

Every year the Department of Health in KwaZulu carries out large-scale control operations against malaria mosquitoes. All buildings in malarious areas are sprayed with 5% DDT, applied at two grams per square metre to the internal surfaces of the walls and roof. In recent years Zulu villagers have increasingly complained that the DDT spraying is making their bedbug problem worse. This claim is supported by a World Health Organization survey in 1967, which showed that, of 32 countries reporting bedbug infestation, 19 considered it a problem in their malaria control projects.[1] However, there has been no published work to date demonstrating a correlation between intradomiciliary spraying of DDT and increased bedbug infestation. Here we report on the results of two surveys which do support such a linkage.

The large majority of rural Zulu huts are constructed of mud walls and thatched roofs, with some dwellings having corrugated iron roofs. Huts were checked visually for adult bugs, nymphs, exuviae or eggs in cracks in walls, in bedding and bedframes, or in other likely harbourages which would indicate present or recent infestation.

In the first survey, huts were checked in 16 areas in KwaZulu between Kwa Mduku in the north (27°51′S; 32° 24′E) and Lake Cubhu in the south (28°50′S; 31°58′ E). Areas and huts were chosen for ease of access from the road. DDT has a very long residual action in mud huts, so huts which had been treated within the past two years were scored as sprayed, and only huts which had never been sprayed were scored as unsprayed. Tables 1 and 2 show respectively that signs of bedbug infestation were detected in 1.2% of unsprayed huts (n = 256) and in 43.8% of sprayed huts (n = 479), the difference being very significant (χ^2 = 147.6; $P < 0.001$).

As only four of the 16 areas surveyed contained both sprayed and unsprayed huts, the possibility existed that some factor linked with the geographical locations of the areas might be affecting the presence or apparent absence of bug infestations. A second survey was carried out which avoided this possible source of bias.

In nine areas of the Ingwavuma and Ubombo districts of KwaZulu, a number of unsprayed huts are left to monitor the occurrence of malaria mosquitoes. These are referred to as controls, and represent unsprayed islands in an otherwise completely sprayed section of the country. Searches for bedbugs carried out in control huts and in a comparable number of nearby sprayed huts gave the results in Table 3. Again, significantly more sprayed huts were infested with bedbugs compared to unsprayed huts (n = 144; χ^2 = 12.1; $P < 0.001$). Newly built huts are less likely to be infested with bedbugs, and may be over-represented in huts reserved as controls. However, even after discounting the 14 control huts less than 9 months old, the result is still significant (χ^2 = 8.6; $P < 0.01$).

Table 1. Results of visual searches for bedbugs in huts in KwaZulu which had not been sprayed with DDT.

District	No. of areas sampled	No. of huts with bedbugs	No. of huts without bedbugs
Ubombo	1	0	7
Hlabisa	2	1	28
Nseleni	7	0	120
Ongoye	1	2	98
Total	11	3	253

Table 2. Results of visual searches for bedbugs in huts in KwaZulu which had been sprayed with DDT.

District	No. of areas sampled	No. of huts with bedbugs	No. of huts without bedbugs
Ubombo	2	70	90
Hlabisa	4	136	151
Nseleni	3	4	28
Total	9	210	269

Table 3. Results of visual searches for bedbugs in huts in nine areas of Ingwavuma and Ubombo districts.

	No. of huts with bedbugs	No. of huts without bedbugs
Sprayed with DDT in last 2 years	22	64
Never sprayed	2	56

It must be emphasized that a negative finding on visually searching a hut for bedbugs is not proof that none is present. However, in both surveys reported here, signs of bedbug infestation have been encountered very much more often in DDT-sprayed huts than in unsprayed ones. One possible explanation of this finding is that widespread and efficient predators and parasites of bedbugs exist among the normal fauna of a mud hut, but they are susceptible to DDT and are not found in sprayed huts. This and other hypotheses await investigation.

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1. Rafatjah H. (1971). The problem of resurgent bedbug infestation in malaria eradication programmes. *J. trop. Med. Hyg.* 74, 53–56.

Usinger (1966)). Overal & Wingate (1976) thought the Stricticimex antennatus they found in a bat cave supported predators because reduviid nymphs were found which had reddish abdomens as if they had consumed engorged bat bugs, and in the laboratory the reduviids eat numerous bugs. Pseudoscorpions and spiders abounded in the cave, but were not seen to feed on S. antennatus.

The above evidence, though circumstantial and largely anecdotal in nature, suggests that predators can be effective in controlling bedbugs. It was therefore decided to undertake a very preliminary observation on the possible role of spiders as predators of bedbugs in the study area. As DDT-sprayed hut walls are often partially or completely replastered by the occupants, which covers the insecticide, (see Section 8.6) spiders can sometimes be found in replastered huts, and collections were made to see if any species were present which might prey on bedbugs.

Materials and Methods

Thirty eight spiders were collected from 43 DDT-sprayed huts in the study area and preserved in 70% alcohol, 20 specimens being identified to family, genus or species level (G. Newlands, pers.comm.*).

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Results

The spiders identified were: Clubionidae; Hersilidae (3); Pholcidae; Pisauridae; Theridiidae (6); Euphrosthenops sp.; Oecobius sp.; Pholcus sp.; Selenops sp.; Theridion sp.; Scytodes thoracica. Very few spiders and limited webbing were noticed in the DDT-sprayed huts, in contrast to unsprayed huts where walls were sometimes festooned with webs and many spiders were seen.

Discussion

The Clubionidae, Scytodes, Selenops, and Theridion are considered likely predators of bedbugs by Dr. Newlands, and were found in Zulu huts. The question therefore arises whether the large spider populations found in huts not treated with DDT would be able to eliminate small numbers of bedbugs carried into huts before extensive reproduction of the bugs could take place. More research is definitely needed on this topic.

8.4 LABORATORY TESTS ON THE SUSCEPTIBILITY OF BEDBUGS FROM KWAZULU TO DDT, DIELDRIN AND FENITROTHION

Materials and Methods

Technical grade insecticides (Feroz, 1968) were dissolved in acetone then an equal volume of silicone fluid grade DC 556 added to make six ml of solution. The insecticide solution was drawn up into a 2 ml syringe and expelled one drop at a

time on to the smooth side of a Whatman's No. 1 filter paper. When drops were made to slightly overlap, starting from the outside of the filter paper and spiraling inwards, 0,6 ml were used up as the centre was reached, and ten filter papers could be prepared.

The area of one side of a filter paper was 0,0064 m-2, and the amount of insecticide to be dissolved was calculated according to the concentration required. Control papers were prepared by treating papers with 0,6 ml of acetone/silicone fluid mixture. Filter papers were treated and dried whilst being supported on a bed of pins. This method is used by the Wellcome Research Laboratories, United Kingdom (A J R Pope, pers.comm., see page 163*).

The following day, each filter paper was cut into four discs of 35 mm diameter and each disc inserted to cover the bottom of a 57 x 35 mm plastic tube. Adult bedbugs caught by hand in Zulu huts were fed in the laboratory on sedated, shaved guinea pigs. Ten insects of the same species and sex were placed into each tube three or four days after feeding. A strain of C. lectularius (SABS) colonized at the South African Bureau of Standards, Pretoria, was similarly fed and sorted into tubes, as were adult progeny of both crosses between SABS and wild-caught C. lectularius.

The bedbugs were exposed to DDT for five days at concentrations of 0,5; 2,0 and 8,0 g m-2, and to dieldrin

for over two days (WHO, 1981) at concentrations of 0,125; 0,5; 1,0 and 4,0 g m-2. One experiment on fenitrothion tested continuous exposure of bugs on 0,25 g m-2 for up to 24 hours, and in the other trial a series of exposure times was used (19, 38, 75, 150 amd 300 minutes) on a concentration of 0,125 g m-2.

In the last experiment, the mortalities following a 24 hour holding period were plotted against time of exposure on probit-log paper for wild-caught C. lectularius, C. hemipterus, SABS strain C. lectularius and the total results of all of the above. Regression lines were fitted using the method of Litchfield & Wilcoxon as described by Swaroop (1966). In this statistical procedure attempts at fitting a line to pass near all the points are assessed by the significance of the sum of chi-squared values arising from the expected values indicated by the candidate line, and the observed values represented by the data points. A number of statistically acceptable regression lines is therefore possible, and no absolute regression coefficient can be calculated for this type of data.

Results

Wild-caught adults of C. lectularius, C. hemipterus and the wild C. lectularius/SABS C. lectularius cross showed survival following exposure to all dosages of DDT, unlike the susceptible SABS strain of C. lectularius (Tables 17 and 18). When exposed to dieldrin-treated papers, wild-caught

bugs of both species had survivors after two days exposure, which never happened amongst the SABS insects (Tables 19 and 20). The susceptibilities of wild and SABS strains of C. lectularius and wild C. hemipterus to fenitrothion were the same (Tables 21 and 22). Straight line regressions could be fitted using the method of Swaroop (1966) for the data arising from the mortality/exposure time tests of wild C. lectularius and C. hemipterus on fenitrothion (Fig. 5, page 178) and for the total results for all bugs tested, but not for the SABS strain (Fig 6, page 179). In the latter case, the data were too heterogeneous for any regression line to fit all the data points.

Discussion

The target dosage rate for the annual anti-malaria intradomiciliary spraying of DDT is 2 g m-2 of active ingregient. The susceptible SABS strain of C. lectularius was unable to survive exposure to even 0,5 g m-2 of DDT on filter paper for three days, whereas a proportion of the wild-caught bedbugs of both species survived 8 g m-2 for five days. This ability to live at high concentrations of DDT was heritable as demonstrated by the Wild/SABS cross C. lectularius which had survivors on the 8 g m-2 DDT after five days. The ability of both bedbug species to flourish in huts annually sprayed with DDT can clearly be ascribed, at least partially, to physiological resistance.

Table 17. Cumulative percentage mortalities of adult male wild-caught C. lectularius and C. hemipterus and SABS strain C. lectularius exposed to DDT impregnated filter papers for five days.

Bedbugs (n)	DDT deposit (g m-2)	% mortality at intervals during exposure				
		1 day	2 days	3 days	4 days	5 days
Wild C. lectularius (10)	Control	0	0	0	0	20
"	0,5	10	10	30	30	30
"	2,0	0	20	20	20	50
"	8,0	0	10	30	50	70
Wild C. hemipterus	Control	0	10	20	20	20
"	0,5	10	10	20	20	20
"	2,0	0	0	0	0	0
"	8,0	10	20	20	20	40
SABS C. lectularius	Control	0	10	10		
"	0,5	40	90	100		
"	2,0	60	100			
"	8,0	100				

Table 18. Cumulative percentage mortalities of adult wild-caught C. lectularius and C. hemipterus, SABS strain C. lectularius and wild/SABS C. lectularius cross exposed to DDT impregnated paper at 8 g m-2 for five days.

Bedbugs (n)				% mortality at intervals during exposure				
				1day	2days	3days	4days	5days
Wild C. lectularius	males	Control	(10)	0	0	0	0	0
"	females	"		0	0	0	0	0
"	male	Experiment	(20)	5	30	30	40	45
"	female	"	(10)	10	30	30	30	30
Wild C. hemipterus	male	Control	(10)	0	0	30	30	30
"	female	"		0	10	20	40	40
"	male	Experiment	(10)	0	0	30	30	30
"	female	"	(20)	20	35	40	45	60
SABS C. lectularius	male	Control	(10)	0	0			
"	female	"		0	0			
"	male	Experiment	(20)	85	100			
"	female	"		100				
Wild/SABS cross	male	Control	(10)	0	0	0	0	0
"	female	"		0	0	0	0	0
"	male	Experiment	(10)	30	50	80	80	80
"	female	"	(20)	5	10	20	25	35

Table 19. Cumulative percentage mortalities of adult male wild-caught C. lectularius, C. hemipterus and SABS strain C. lectularius exposed to dieldrin impregnated paper for three days.

Bedbugs (n)	dieldrin deposit (g m-2)	% mortality at intervals during exposure		
		1 day	2 days	3 days
Wild C. lectularius (10)	Control	0	0	10
"	0,125	20	90	90
"	0,5	70	90	90
"	1,0	90	90	90
"	4,0	90	90	90
Wild C. hemipterus (10)	Control	0	10	20
"	0,125	50	100	
"	0,5	70	90	100
"	1,0	90	90	90
"	4,0	90	100	
SABS C. lectularius (10)	Control	0	0	0
"	0,125	10	70	100
"	0,5	40	100	
"	1.0	90	100	
"	4,0	100		

Table 20. Cumulative percentage mortalities of adult male wild-caught C. lectularius, C. hemipterus and SABS strain C. lectularius exposed to dieldrin impregnated paper at 1 g m-2 for three days.

Bedbugs (n)			% mortality at intervals during exposure		
			1 day	2 days	3 days
First experiment					
Wild C. lectularius	Control	(10)	10	10	10
"	Experiment	(20)	15	60	85
Wild C. hemipterus	Control	(10)	0	10	10
"	Experiment	(20)	70	95	100
SABS C. lectularius	Control	(10)	0	0	
"	Experiment	(20)	80	100	
Second experiment					
Wild C. lectularius	Control	(10)	0	10	10
"	Experiment	(20)	75	80	100
SABS C. lectularius	Control	(10)	0	0	
"	Experiment	(20)	95	100	

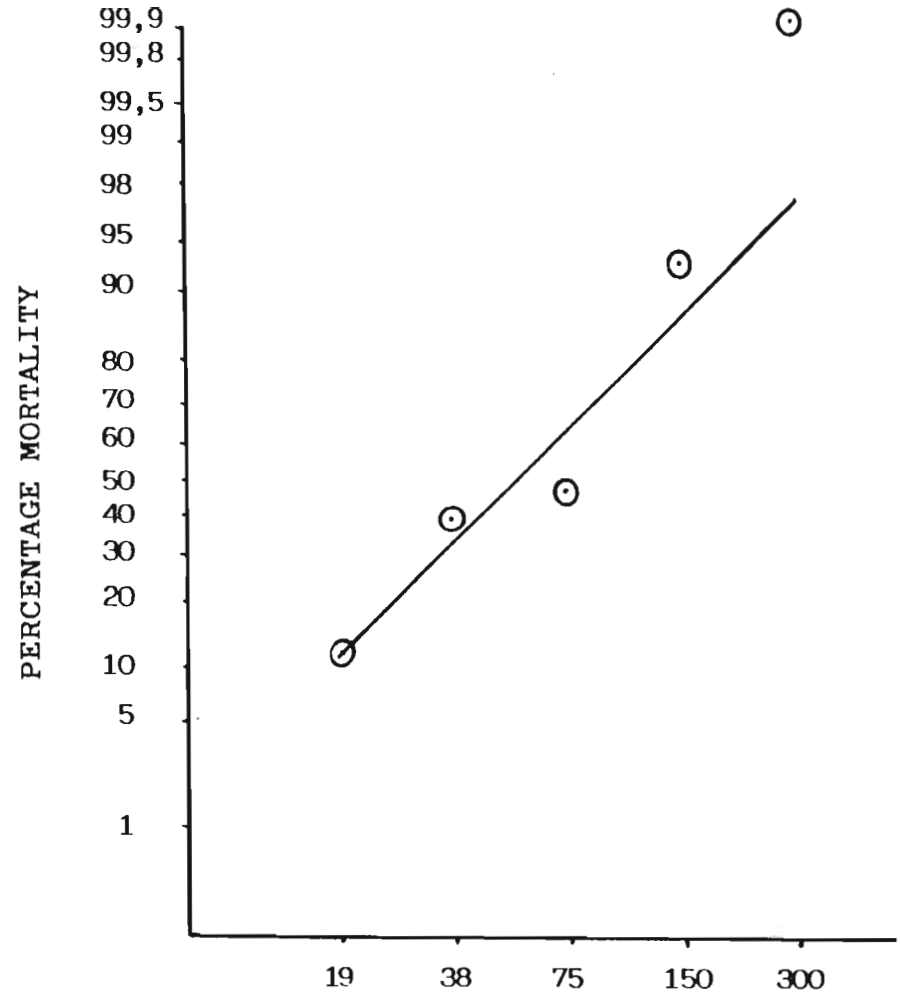
Table 21. Cumulative percentage mortalities of adult male wild-caught C. lectularius, C. hemipterus and SABS strain C. lectularius exposed to fenitrothion impregnated papers at 0,25 g m-2 for 24 hours.

Bedbugs (n)			% mortality at intervals during exposure		
			3 hours	7 hours	24 hours
Wild C. lectularius	Control	(10)	0	0	
"	Experiment	(10)	20	100	
Wild C. hemipterus	Control	(10)	0	0	
"	Experiment	(10)	80	100	
SABS C. lectularius	Control	(10)	0	0	0
"	Experiment	(10)	0	70	100

Table 22. Percentage mortalities of adult male wild-caught C. lectularius, C. hemipterus and SABS strain C. lectularius after exposure to fenitrothion-impregnated paper at 0,125 g m-2 for up to five hours. (* Female bugs used in one replicate).

Bedbugs	No. of replicates (each with 10 bugs)	length of exposure (minutes)	% mortality 24hrs after exposure
Wild C. lectularius	5	Control	0
"	4	19	12,5
"	4	38	40,0
"	4	75	47,5
"	6	150	93,3
"	4	300	100
Wild C. hemipterus	4	Control	2,5
"	4	19	32,5
"	4	38	47,5
"	4	75	87,5
"	5	150	98,0
"	4	300	100 *
SABS C. lectularius	5	Control	0
"	4	19	37,5
"	4	38	47,5
"	4	75	62,5
"	6	150	100
"	4		

Fig. 5 Percentage mortalities of wild-caught C. lectularius (top) and C. hemipterus (bottom) following 24 hours holding after exposure to paper treated with fenitrothion at 0,125 g m-2 for 19,38, 75, 150 or 300 minutes, plotted on probit-log paper.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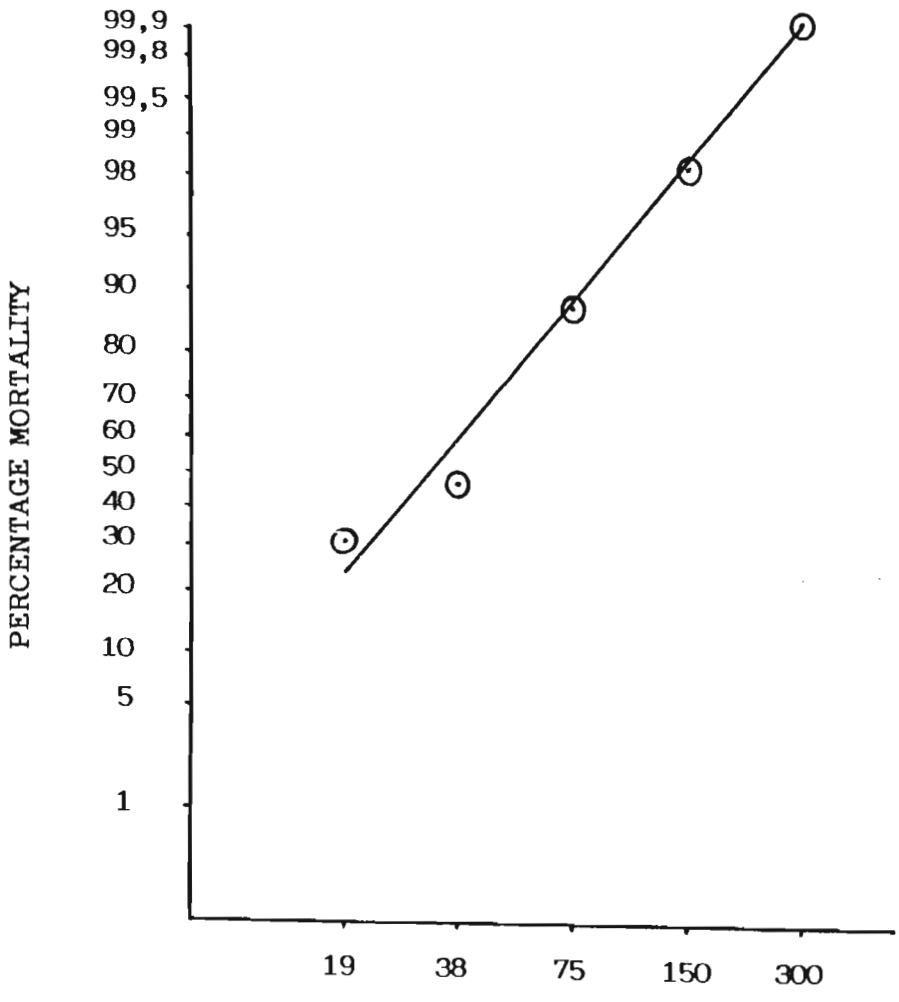
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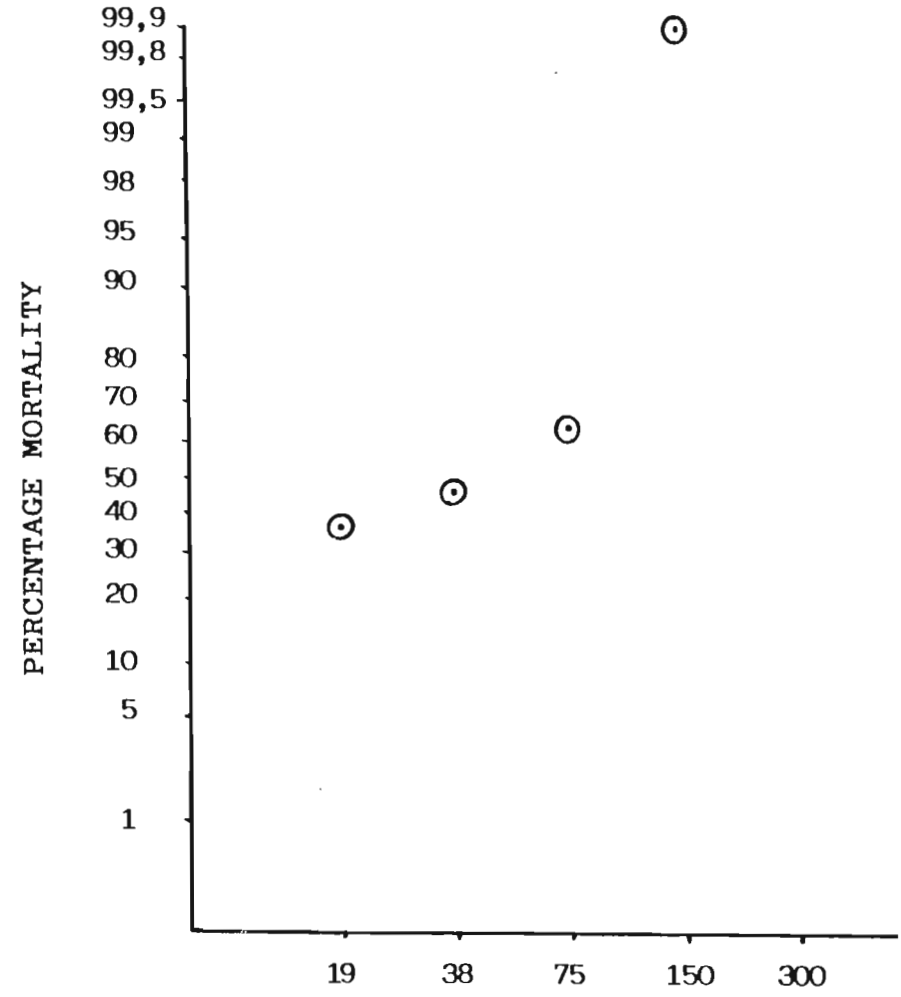
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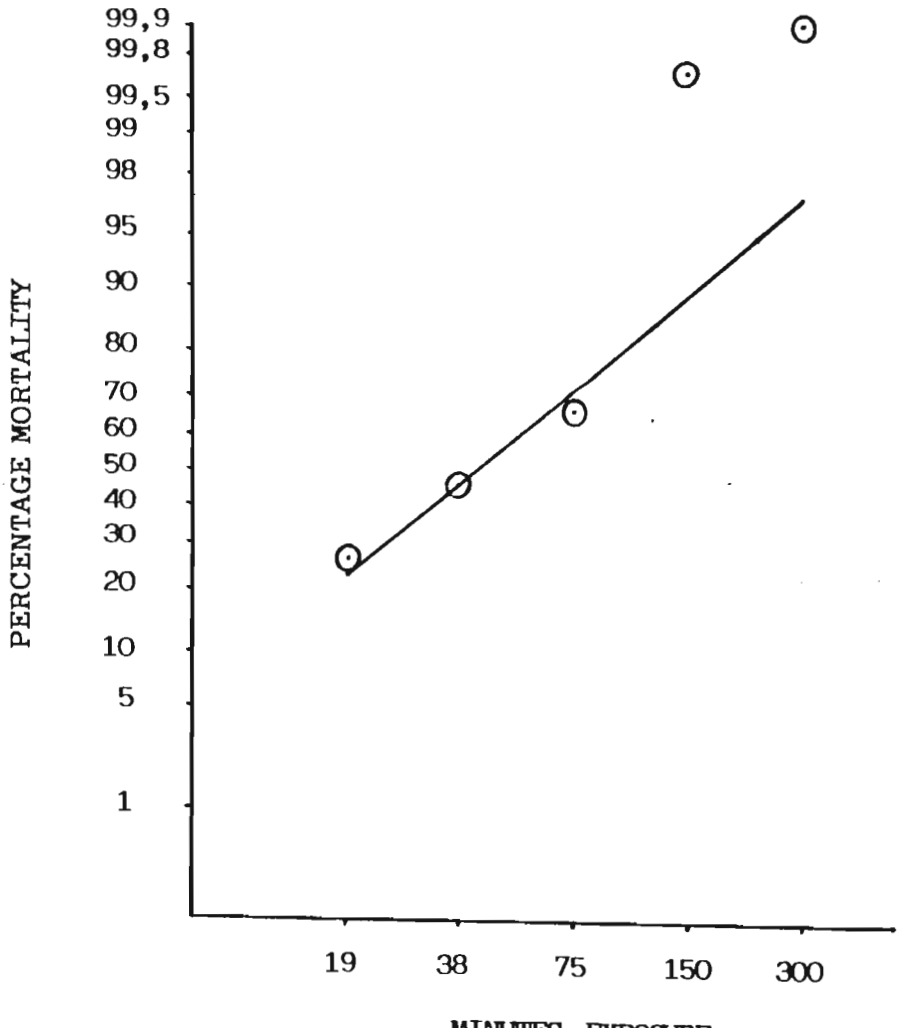
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Whitehead (1962) found resistance to both DDT and dieldrin/BHC in the C. lectularius he tested in Johannesburg. In the present work, one or two wild bugs of both species survived exposure to dieldrin concentrations which swiftly killed all the SABS strain. Dieldrin/BHC resistance may therefore be present in the populations, but not at as high a frequency as DDT resistance. Dieldrin and BHC were banned from use in South Africa by the Government Gazette of 1st of May, 1981, but BHC had been used intermittently between 1946 and 1962 for malaria control in Natal (D M Eckard, pers. comm.*; de Meillon et al., 1977). Selection for DDT resistance continues to the present day, huts being sprayed annually with this insecticide.

The recommended dosage rate at which fenitrothion should be sprayed for the control of bedbugs is 1 g m-2. The swift death of all bugs exposed to 0,125 g m-2 suggests that this insecticide should be effective against wild bedbugs in Natal and KwaZulu. The SABS strain was no more susceptible to fenitrothion than the wild bugs of each species, which both showed a linear mortality/exposure relationship on log-probit scales indicative of homogeneous susceptible populations. The reason for the heterogeneous data arising from the SABS strain of C. lectularius is not known but was probably the result of random variables.

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8.5 FIELD TRIALS OF THE INSECTICIDES BENDIOCARB, DELTAMETHRIN AND FENITROTHION FOR THE CONTROL OF BEDBUGS

Three insecticides available in South Africa have residual lives long enough on mud wall surfaces to have been considered for malaria control in other countries. A long residual action is desirable when eliminating bedbug infestations since during the egg stage bugs may not contact the insecticide for days or weeks, depending on the temperature. Also, an active insecticide residue will kill any bugs being brought in, and thereby prevent reinfestation.

Deltamethrin, a synthetic pyrethroid, was highly effective against Anopheles arabiensis for over two years at a dosage rate of 5-6 mg m-2 on a mud wall (Taylor et al., 1981). In Nigeria, bioassays on mud walls showed excellent residual effect for over three months (Rishikesh et al., 1978). Bendiocarb, a carbamate, sprayed at 0,4 g m-2 gave 45 days effective persistence on mud in Iran (Eshghy et al., 1979). Fenitrothion, an organophosphorus compound, killed over 80% of An. gambiae on bioassay for five months after being sprayed at 2 g m-2 on mud walls (Fontaine et al., 1975). Bhatnagar et al. (1974) found fenitrothion at 1 g m-2 on a sorptive surface gave eight weeks residual action against An. cullicifacies in India. All three insecticides are registered for use against bedbugs in South Africa.

Materials and methods

Mud-walled Zulu huts were searched for bedbugs in the usual hiding places of beds, furniture and cracks in walls. The internal dimensions of the walls and roofs of heavily infested huts were measured and then sprayed with a test insecticide. The chemicals were all in wettable powder formulation, with deltamethrin also tested in a microencapsulated, slow-release form. The products used were Ficam, which contains 80% bendiocarb, Rescol, containing 40% fenitrothion, and the deltamethrin compounds which included 2,5% of active ingredient deltamethrin.

A Hudson Xpert pressure pump was used fitted with a 8002 T nozzle (80 degree angle of spray, 0,02 inches (0,5mm) aperture). The concentration of insecticide per litre of aqueous solution was calculated so that the target dosage in g m-2 would be approximated when applied at the same speed and distance from wall to nozzle as practised by Department of Health teams when spraying DDT. Target dosage rates were 0,4 and 1,0 g m-2 for bendiocarb and fenitrothion respectively. A dosage rate of 15 - 25 mg m-2 was recommended by the suppliers of deltamethrin. A special formulation of fenitrothion with enhanced suspensibility was also tested to see if bedbug and malaria control could be carried out by one spraying using a mixture of the two insecticides (666 g 75% active ingredient DDT and 625 g 40% active ingredient fenitrothion in 10 litres of aqueous solution).

White spray sheets were laid on the floor, under furniture, to catch dead bugs, and bed frames, mattresses and furniture were sprayed lightly using a second pump. The dosage rates employed were calculated allowing for the percentage active ingredient in each chemical used. The following day sheets were removed and dead bedbugs were collected into labelled tubes. Back in the laboratory the insects were counted and scored to species using morphological criteria (Usinger, 1966; Chapter Two).

Bioassays were carried out on sprayed mud walls using plastic cones (WHO, 1960) designed for mosquito work. They were adapted to accommodate the irregular surfaces of mud walls by gluing 5 mm thick foam rubber to the base rims. Ten wild adult bedbugs caught the previous or same day were tipped into each cone and exposed to the mud wall for five hours, the cones being attached to the walls by pins. Control exposures were carried out on unsprayed mud walls. Bugs were then brushed off the walls and held in plastic tubes containing cardboard strips for the insects to sit on. Mortality was scored after 24 hours, and corrected test mortalities were calculated using Abbott's formula when control mortalities were between 5 and 20% (Swaroop, 1966).

One month or more after treatment, huts were again examined for bedbug infestation. Visual inspection was sufficient to confirm the presence of bedbugs in the case of one insecticide trial, but on all other occasions the knockdown

insecticide Insectigas (registered trademark of Coopers L2131 Act 36 of 1947) was used. The active ingredient of Insectigas is dichlorvos at a concentration of 93g/kg which is dispensed under pressure from a cylinder containing liquid carbon dioxide to give a highly penetrating aerosol (Newberry et al., 1986). A burst of three to five seconds was delivered, depending on hut size, with doors, windows and other openings closed as far as possible. Spray sheets were laid on the floor, under furniture, to catch dead bugs which were collected after an interval of at least two hours, and later counted and sorted into species.

Results

Tables 23 and 24 record the details of the trials and post-spraying monitoring. Since the survival of test insects after exposure to insecticide is still important (WHO, 1981), two bioassay results are included in which control mortalities exceeded 20%. Table 25 shows the knockdown of bedbugs two hours after spraying with Insectigas with the accumulative total after 24 hours.

Discussion

The methods used in some published reports of field trials of insecticides for bedbug control made their results difficult to interpret. Whitehead (1962) tested two insecticides in a block of rooms where movement of bedbugs from one to the other was very probable. His trap board could prove the presence of bedbugs, but the sensitivity of

Table 23. Results of field trials of three insecticides used for bedbug control monitored by detection of persistent or renewed infestations of C. lectularius (L) or C. hemipterus (H).

Insecticide and average dosage rate in g or mg m-2	No. of infested huts sprayed and species present	Interval between spraying and monitoring (days)	No. of huts with post-spraying infestation/ total
Bendiocarb 0,38 g	17 (15L + 2H)	34	11/12 (9L + 2H)
Deltamethrin 20,17 mg wettable powder	5 (4L + 1 L&H)	70	1/5 (L)
Deltamethrin 17,83 mg microencapsulated	11 (10L + 1 L&H)	70	6/9 (L)
Fenitrothion 1,06 g	11 (H)	62	0/11
1,47 g	10 (2L + 4H + 4 L&H)	35	0/9
0,87 g + 1,74 g DDT	8 (L)	84	2/8 (L)

Table 24. Results of bioassays using wild-caught bedbugs on mud walls of huts sprayed for the control of C. lectularius (L) and C. hemipterus (H). The exposure time was 5 hours, mortality was scored after 24 hours

Insecticide and average dosage rate in g or mg m^{-2}	Interval between spraying and monitoring	% mortality on bioassay (n)		X^2 value and significance of difference between test and control		Corrected % test mortality
		Test	Control	X^2	Significance	
Bendiocarb 0,38 g	34	L: 61(103)	53(59)	1,15	N.S.	-
Deltamethrin 20,17 mg wettable powder	36	L: 15(40)	10(20)	0,29	N.S.	6
Deltamethrin 17,83 mg microencapsulated	35	L: 66(57)	13(40)	27,95	P 0,001	61
Fenitrothion 0,54 g	33	L: 15(73)	9(47)	1,12	N.S.	7
1,06 g	33	L: 90(44)	9(47)	61,78	P 0,001	89
1,06 g	62	L & H: 81(109)	10(70)	85,63	P 0,001	79
1,47 g	35	H: 77(77)	30(43)	24,74	P 0,001	-
0,87 g + 1,74 g DDT	35	H: 100(59)	18(50)	77,55	P 0,001	100
	84	L: 76(103)	11(46)	54,21	P 0.001	73

Table 25. Knockdowns of C. lectularius using Insectigas: collections made from two huts after 2 hours, with cumulative totals presented for the 24 hours catch.

	Adults		Nymphal stages						
	Male	Female	V	IV	III	II	I	Total	%
Hut 1									
2 hours	30	41	49	39	25	29	66	279	70
24 hours	60	61	72	55	32	48	76	404	100
Hut 2									
2 hours	31	42	100	172	194	298	598	1432	57
24 hours	58	83	208	310	359	500	1005	2523	100

of the method was unknown. Shetty et al. (1975) dropped charpoys on to the floor and counted the dislodged bedbugs to assess survival of infestations following the spraying of fenitrothion, chlorpyrifos and malathion in Indian barracks. Varma & DuttaGupta (1983) used the same method to monitor the effectiveness of malathion and fenitrothion. Once again, the sensitivity of their assessment was not discussed.

In the present work it has been shown that about 70% of a C. lectularius infestation is knocked down 24 hours after a treatment with Insectigas (see page 80). Table 25 records that over 50% of the 24 hour knockdown is dead two hours after spraying, which makes collection after two hours at least 35% efficient.

It is impossible to prove that no bedbugs remain in a hut, or to distinguish between resurgence of a surviving post-spraying infestation and reinfestation from an external source. One cannot therefore gain an idea of the residual life of an insecticide by counting weeks or months free from bedbugs because reintroduction of the insects may not be occurring. The bioassays carried out in the present work have given an indication of the residual life of the insecticides used. A low lethal effect after five weeks was demonstrated for deltamethrin wettable powder and fenitrothion at 0,5 g m-2, and very high residual activity of fenitrothion sprayed at over 1,0 g m-2, for up to 12 weeks expecially when mixed with DDT.

On the basis of the bioassay results and post-spraying knockdowns, fenitrothion was considered the most promising insecticide for bedbug control in the study area. Only three bugs were found 84 days after spraying in each of the two huts treated with the fenitrothion and DDT mixture., and probably represent a reinfestation. Eradication is nearly always, perhaps always, initially achieved. This insecticide was therefore recommended in 1985 to the Health Departments for use in bedbug control in mud-walled huts, and has been used with complete success to date (see Section 8.7).

Resistance to the organophosphorothioates (P=S compounds), the group of chemicals which includes fenitrothion, has been reported in C. lectularius in Israel (Barkai, 1964; Feroz, 1971) where malathion and diazinon were used for control, and in the USSR (WHO, 1980). Shetty et al. (1975) suspected malathion resistance in C. hemipterus in India, though fenitrothion was found to be effective. Feroz (1969) concluded that the resistance of the Israeli bedbugs was not based on a simple mechanism relating to the structure of the insecticides he tested. The bugs' susceptibilities to eleven organophosphorothioates varied considerably and no resistance was found to the organophosphates, carbamates, or organochlorines. A single autosomal recessive gene was shown to determine the organophosphorothioate resistance.

Should resistance to fenitrothion appear in the bedbugs of Natal and KwaZulu, the spread of the gene will be less rapid

because of its recessive nature. Although DDT resistance is already well established, it appears that susceptibility to carbamates will not be affected, leaving this group of insecticides for future use. In this respect, however, the results for bendiocarb were not promising.

8.6 THE EFFECT OF BEDBUG CONTROL ON THE REPLASTERING OF THE WALLS OF MUD HUTS BY THE OCCUPANTS

Every year the Departments of Health of KwaZulu and South Africa spray the internal surfaces of walls and roofs of buildings in malarious areas with DDT as a control measure against vector mosquitoes. The DDT is applied at 2 g m-2, which fails to kill bedbugs (Chapter Four) due to their high degree of resistance to this insecticide (Section 8.4). DDT is an irritant insecticide, and Zulus living in infested huts often complain that bedbugs bite them more frequently following DDT spraying. In response to the increased nuisance, many hut occupants living in mud-walled huts replaster over the fresh DDT deposit. However, there are also reasons why hut occupants may replaster which are not related to bedbug nuisance. To some rural people, the white colour of the DDT deposit is unpleasant. Festive occasions or the home-coming of a relative may also instigate the replastering of a hut for decorative purposes, or walls may become so badly cracked that they need repairing. It was therefore not known to what extent replastering was carried out because of bedbug infestation. Replastering is of

considerable concern to the Health Departments because it negates their malaria control effort to some extent. It was therefore necessary to find out to what degree bedbug control could reduce the level of replastering.

Materials and methods

One survey was carried out in the study area of KwaZulu north of 27 45′ (Fig. 7), and a second survey was conducted in the Nkundusi region between 28 S and 28 15′ S (Figs. 1 and 2 of Newberry et al. (1987), pages 53 and 54 of Chapter Three). DDT-sprayed huts were checked for current infestation by searching harbourages for nymphal and adult bedbugs. Infestation prior to earlier spraying with fenitrothion (Section 8.5) was noted when exuviae or empty egg cases could be seen but not live bugs. Replastered huts were recognized because there was no trace of DDT on the walls though the white deposit could be seen on the ceilings.

Results

Table 26 and 27 show the impact of bedbug control on replastering rates found during the survey conducted in the northern areas. Table 28 records replastering rates in the Nkundusi area in two areas where bedbug control was, and was not, carried out respectively. Table 29 presents the month by month replastering of huts at Nkundusi, comparing successfully treated, previously infested, huts with huts which had not been sprayed and were still infested.

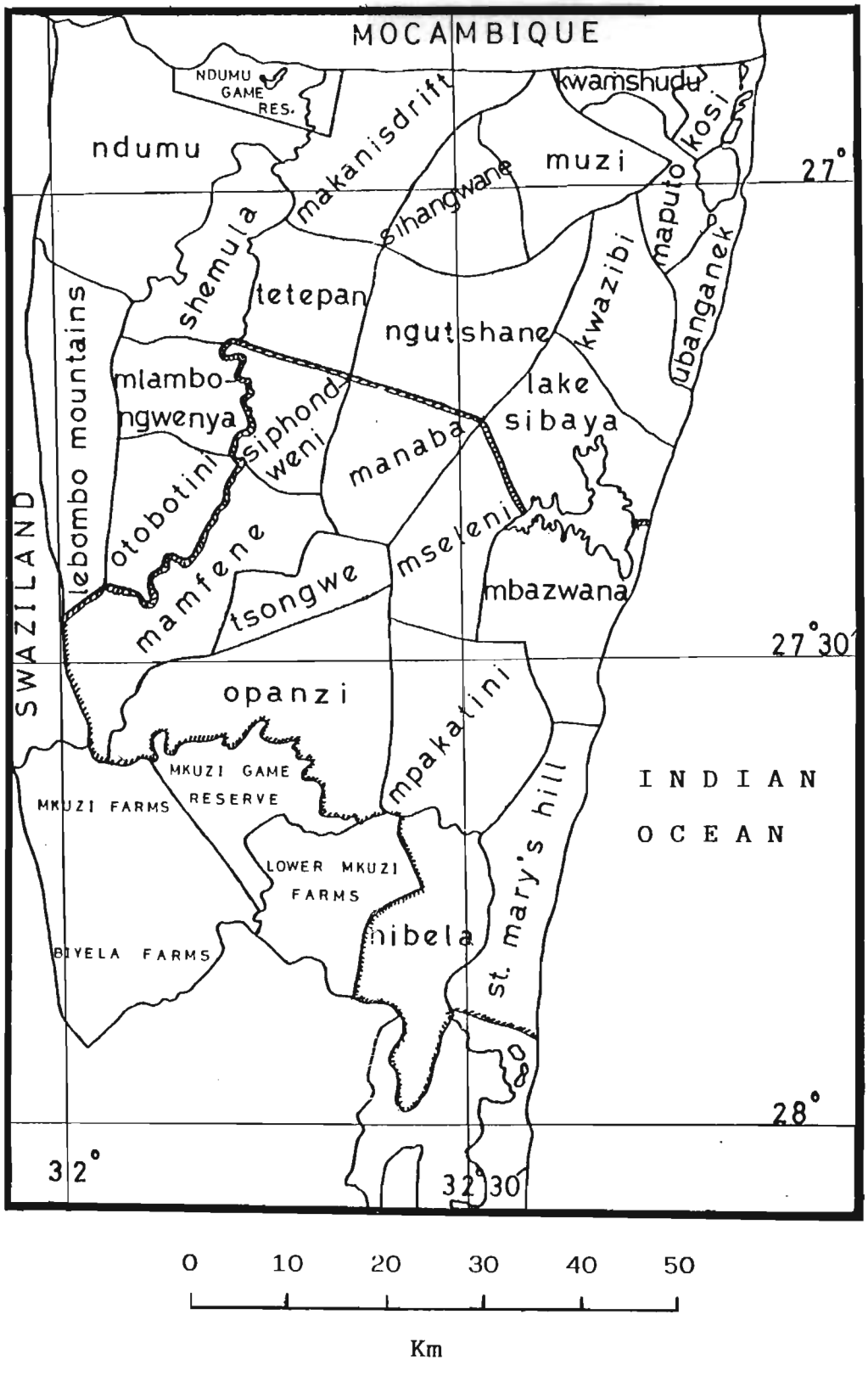

Fig. 7 The northern study area

Table 26. Replastering of huts and bedbug infestation in relation to bedbug control in Ingwavuma and Ubombo Districts of KwaZulu.

Infestation and replastering status of huts	(Area) Ophansi	Mamfene	Siphondweni	Tetepan	Shemula	Sihangwane & Ngutshane	Ndumu	Total	%
a) No history of infestation Not replastered	44	94	44	66	30	70	19	367	77,10
b) No history of infestation Replastered	11	30	6	14	8	12	28	108	22,90
c) Bedbugs eradicated Not replastered	29	18	29	13	14	24	18	145	81,92
d) Bedbugs eradicated Replastered	4	5	3	1	6	2	11	32	18,08
e) Infested. Not replastered	3	4	2	7	2	2	0	20	50,00
f) Infested. Replastered	0	7	2	7	1	0	3	20	50,00

Table 27. Analysis of data in Table 26 comparing the frequency of replastering in mud-walled huts in relation to bedbug infestation.

Comparison of replastering	Chi-squared value	Degrees of freedom	Significance
Previous infestation/ never infested (c & d)/(a & b)	1,66	1	N.S.
Current infestation/ previous infestation (e & f)/(c & d)	18,25	1	P<0,001
Current infestation/ never infested (e & f)/(a & b)	14,68	1	P<0,001

Table 28. Replastering of DDT-sprayed huts and bedbug infestation in relation to bedbug control in the Nkundusi area of KwaZulu.

Area where no bedbug control was carried out

Infestation status of huts	No. of huts replastered	No. of huts not replastered	% replastered
Current	3	21	12,5
Previous	3	7	30,0
Never infested	10	38	20,8

Area where bedbug control was carried out

Infestation status of huts	No. of huts replastered	No. of huts not replastered	% replastered
Current	8	5	61,5
Previous	13	8	61,9
Never infested	11	39	22,0

Table 29. Number of DDT-sprayed huts replastered per month which had been deinfested by treatment with fenitrothion, compared with huts which were infested and unsprayed.

	Replastering	
	Previously infested huts (n = 12)	Infested huts (n = 12)
Jan.	0	2
Feb.	3	5
Mar.*	4	6
Apr.	5	7
May	5	7
Jun.	6	7
Jul.	6	7
Aug.	6	7

* One previously infested hut was reinfested in March, but not replastered.

Discussion

The survey encompassing the northern districts of KwaZulu (Table 26), showed that the high rate of replastering in bedbug infested huts (50%) was significantly reduced in huts treated with fenitrothion (18%) to a level similar to that found in uninfested huts (23%). This suggests that replastering occurs in about 20% of huts for reasons unrelated to bedbugs. The Nkundusi survey did not show any relationship between bedbug infestation and the rate of replastering (Table 27), whether the bugs had disappeared without spraying or following treatment with fenitrothion. Again, the replastering rate in huts in which bedbugs had never been a problem was approximately 20%, but removal of infestations did not bring the replastering rate down in infested huts. The month by month assessment (Table 28) also showed that no difference in replastering rates was brought about by bedbug control in the Nkundusi area. The reasons for this must be sociological, and are possibly related to the effectiveness of health education. However, the ability of bedbug control to greatly reduce the level of replastering, and hence the obstruction to malaria control, has been demonstrated in the northern areas, and similar results in all areas are therefore possible.

8.7 REINFESTATION

Reinfestation is brought about by passive dispersal, and the introductory remarks of Chapter Three are therefore

relevant. A hut treated with a residual insecticide is vulnerable to reinfestation when the deposit loses its lethal effect. The interval of time between spraying and reinfestation therefore depends on the length of active life of the insecticide residue, and the period following this before bedbugs are reintroduced into a building. The risk of reinfestation will be proportional to the number of visits made by people from infested houses, the amount of luggage they bring with them, and the length of their stay, particularly if it is overnight. As such, buildings such as hostels used by students from third world countries (Story, 1984) and holiday accommodation (Cornwell, 1974) are very likely to become repeatedly reinfested. In the present study, huts sprayed with fenitrothion were monitored for infestation to determine whether the treatment was effective, and to assess the rapidity of reinfestation.

Materials and methods

Forty one infested huts in the Sihangwane (11), Opansi (12 and 8) and Mfekayi (10) areas (Figs. 1 and 2 of Newberry and Mchunu (1989) pages 56 to 58 of this thesis) were sprayed with fenitrothion under the author's supervision. The huts were searched one month later for surviving or renewed infestation, then the huts in each area were monitored at different times using Insectigas knockdown (Section 8.5) for up to 15 months. In 1986, 57 huts in the Mamfene (15 and 15), Siphondweni (6), Manaba (6 and 3), Ngutshane (8), Sihangwane (3), and Tetepan(1) areas (Fig. 7 page 192) which

had been treated with fenitrothion by the Department of Health, KwaZulu, on the request of the occupants, were monitored for reinfestation by visual search for up to nine months. Thirty infested huts which had been similarly treated for bedbugs in the Nkundusi area (Fig. 2 of Newberry and Mchunu (1989), page 57 of this thesis) were monitored by visual assessment for reinfestation at five and 15 months. The first study was longitudinal and cross sectional in that the same huts were sampled more than once, but at varying times in the different areas. The latter two studies were cross sectional only, because sets of huts were scored only once for reinfestation. A further cross sectional survey involving huts over the whole study area was conducted in 1987/8 involving huts which had been sprayed by the KwaZulu Department of Health.

Results

Table 30 shows reinfestation which occurred after huts had been treated at the owners' request in 1986. Table 31 shows results of surveys conducted over the whole study area after extensive bedbug control had been carried out during 1987 and 1988. The regression of reinfestation rate on time found in the 1986 work (Fig. 8, page 201) is significantly different from zero ($b = 2,98$; $y = 2,98x - 0,37$; $t = 7,73$; d.f. $= 2$; $P < 0,05$). This relationship also differs significantly from zero in the 1987/8 surveys ($b = 1,15$; $y = 1,15x - 4,07$; $t = 14,16$; d.f. $= 1$; $P < 0,05$) and the two regression coefficients are significantly different from

Table 30. Reinfestation of huts treated with fenitrothion: 1986 survey

Group 1) Monitored by Insectigas knockdown

Group 2) Monitored by visual search (northern areas only)

Group 3) Monitored by visual search (Nkundusi area only)

	Percentage reinfestation (n) at intervals (months) after spraying fenitrothion			
Group	2 - 3	4 - 6	7 - 9	15
1	7,1 (28)	18,2 (11)	28,6 (7)	43,5 (23)
2	0 (24)	13,8 (29)	22,2 (18)	-
3	-	23,3 (30)	-	43,3 (30)
Total	3,8 (52)	18,3 (71)	24,0 (25)	43,4 (53)

Table 31. Reinfestation of huts following treatment with fenitrothion:
1987/8 survey

Time after spraying	Previously infested huts	Reinfested huts	Percentage reinfested
3 - 7 months	417	6	1,4
12 - 16 "	40	5	12,5
22 - 24 "	136	30	22,1

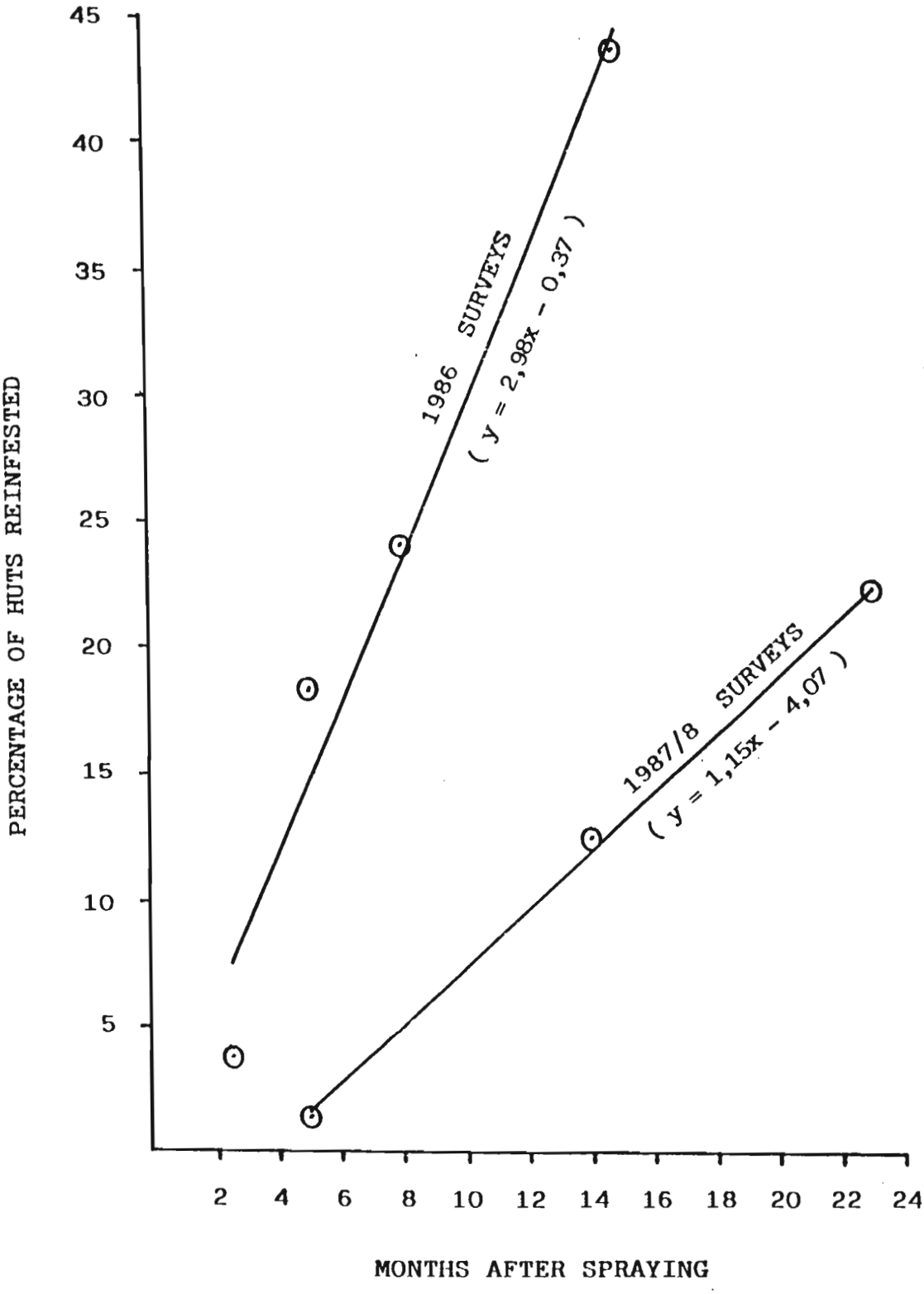

Fig. 8. Percentage reinfestation with time in Zulu huts cleared of bedbugs by the spraying of fenitrothion

each other ($t = 4,60$; d.f. = 3; $P < 0,05$).

Discussion

Fenitrothion sprayed at 1 g m-2 is effective against bedbugs for over two months (Table 24 page 183) so the low infestation rates noted (3, 8 and 1,4%) up to three months after spraying (Tables 29 and 30) are assumed to represent reinfestation rather than the resurgence of populations surviving the spraying. Opportunities for reinfestation from local untreated huts were higher in 1986, when limited bedbug control had been undertaken on a large scale, than in 1987/8 when the cumulative effect of bedbug control operations was greater. The significantly lower rate of reinfestation noted in the later years indicates that reinfestation from local sources is important, though no doubt some reintroduction of bugs occurs when people visit from far away eg. when men return for holidays having lived in hostels.

If all huts were equally at risk, one would expect a rising curve of their reinfestation with time as reinfestation opportunities for uninfested huts increase with the growing percentage of huts harbouring bugs. However, if some huts, where guests frequently stay over night, were at higher risk of reinfestation in comparison to others where few or no people visit, one would expect a falling curve of reinfestation rate with time among the huts, reflecting this sociological variable. The apparent linear relationship

between reinfestation rate and time is perhaps a result of a combination of these two trends.

As bedbug control continues on a recurrent basis in KwaZulu, the reinfestation rate should drop from the levels recorded during 1987/8 as local reservoirs of bedbugs are eliminated. The cost of bedbug control is therefore likely to fall to a baseline dictated by the rate of reinfestation coming from outside the area. The benefits in terms of the goodwill of the rural population and the reduction in replastering over DDT deposits will probably ensure the continued expenditure on bedbug control by health authorities.

CHAPTER NINE

COMMENTS AND SUGGESTIONS FOR FUTURE RESEARCH

There are no known absolute criteria for differentiating C. lectularius from C. columbarius, since the chromosome numbers of the two species may be the same and their morphology is too similar to allow the certain identification of individuals. The low production of eggs resulting from the female C. columbarius x male C. lectularius cross (Ueshima, 1964) indicates a degree of incompatibility between the two species, but cannot be used as a tool for scoring suspected mixed infestations. More detailed morphological or cytological work may reveal reliable distinguishing characteristics between the two bugs, without which their taxonomic status is unlikely to be resolved (see section 9.6). The "animal house C. lectularius" of Johnson (1939), intermediate in the HW/3A ratio between C. lectularius and C. columbarius, remains an enigma, possibly representing a third species of the Lectularius group. Laboratory crosses between "animal house" specimens and the two currently recognized bug species may reveal reproductive isolation in one or both directions and indicate the genetic affinities of this intermediate bug type.

The ability to morphologically distinguish C. lectularius from C. hemipterus at all stages of the life cycle has a practical use should one of the species develop an

insecticide resistance, or demonstrate an important vectorial capacity, not shown by the other. Control decisions can be made on the basis of a sample containing only pre-adult specimens.

Surveys of the frequency of bedbug infestations over large areas were carried out by Markhula and Tiittanen (1970) in Finland, by sending written enquiries to relevant officials and individuals. Not all their enquiries received replies and expert identification of insects was not always possible, so this method was of limited value. Cornwall (1974), compiled his survey of Britain from the records of Rentokil field staff. This method was therefore passive, relying on people reporting infested properties to the company. The surveys of Lewis (1949) in the Sudan were based partly on his own active collections and partly on specimens submitted to him, but few insects were collected from any one place. Mercuria (1967) also did not collect sufficient bedbugs in Ethiopia to draw firm conclusions about distribution.

The present study therefore is unique in that intensive, house to house searches were made over a large area inhabited by both species of bedbug. Within the limitations of the accuracy of visual search for the detection of bedbug infestation, baseline data were collected showing the frequency of infestation of huts by the two species in an area apparently climatically well suited to both of them.

Huts infested by both species were studied over time, and the phenomenon of species replacement observed and documented. The study area was ideal in that it supported large numbers of both bedbug species and it was inhabited by a rural population amenable to the intrusions of bedbug survey work. Since most of the study area has now been subjected to effective bedbug control using fenitrothion, a "natural" situation no longer exists for scientific observation. If other "untouched" places remain elsewhere, more research of a similar kind into the distribution of the bedbug species in areas of sympathy would be useful to compare with the results presented in this thesis.

The finding that wild C. hemipterus lays as many eggs as wild C. lectularius at temperatures averaging 22,5 degrees C to 25 degrees C contradicts the laboratory results presented in this work and that of other authors (Omori, 1941; Walpole 1988a). This questions the relevancy of laboratory comparisons of the two species, which can easily be influenced by different degrees of success in adapting to laboratory conditions. The rate of development of the two species could only be compared under natural conditions by monitoring following the release of bugs at the first nymphal stage into a human habitation. Ethical considerations would preclude this, so non-human hosts would have to be used. However, any interaction between bugs and the uniquely human aspects of host sleeping behaviour, e.g. the use of blankets, would be lost. In general, it appears

that only field studies will add to our knowledge of the relative advantages one species has over the other in their natural environment.

Bedbugs can build up to very large populations, sometimes involving thousands of nymphs and adults, in individual Zulu huts. By virtue of their numbers and apparent restriction of movement to within buildings, the bugs could be very dangerous vectors of hepatitis B virus should an infected person share a hut with uninfected visitors and vice versa.

It would be very useful to be able to accurately assess the size and composition of bedbug populations without killing the insects. An aercsol spray which had a flushing and temporary knockdown effect could be ideal. Once again, the ethics of repeatedly studying a bedbug population in a human habitation without eradicating it, would be questionable.

It has been shown in this work that male C. hemipterus do mate with female C. lectularius when living freely in Zulu huts, and regardless of which species is the more abundant. The laboratory results show that such cross mating could be disadvantageous to C. lectularius in mixed infested huts when C. hemipterus predominates and the field data presented in Chapter Three suggest that species replacement occurs. However, the reciprocal cross, male C. lectularius x female C. hemipterus, has not been studied in depth, and deserves attention. Although deleterious effects on female C.

hemipterus, as regards fertile egg production and longevity, are not obvious, they might exist to some extent. The frequency of this interspecific cross in the wild remains unknown because a massive visible immune reaction does not appear to occur in the female C. hemipterus as is the case in female C. lectularius mated by male C. hemipterus. Possibly more subtle signs of interspecific mating are found in female C. hemipterus which would enable the scoring of cross-mating in wild mixed infestations.

Only studies of mixed infestations under natural conditions will decide whether or not C. lectularius and C. columbarius are distinct species. If the two types can coexist at the same locality without the production of hybrids and introgression of genes leading to the formation of a single bug population, they are good biological species. The same criteria apply, in this case, for the Recognition Concept of species since the two bug types are interfertile and compatible fertilization systems would lead to hybridization. The latter species concept may encounter difficulties concerning whether a bird's nest in a house is as "natural" an environment as one in a tree, and thus a decision on the specific status of the two bugs in terms of the Recognition Concept may be impossible.

To decide whether C. lectularius and C. hemipterus are the same Recognition Species, it must be clear whether interspecific mating in one direction only (male C.

hemipterus x female C. lectularius) is considered sufficient to denote common fertilization systems. Given fully compatible post-mating systems, such mating would bring about introgression and merging of populations, and a sharing of a common gene pool. If fertilization systems end at syngamy, how often must syngamy be achieved for the fertilization systems to be regarded as common? If DDT-sprayed huts are not considered "natural" (Walpole, 1988a), how close to the presumed primordial habitat of bedbugs must the environment approximate before it is considered suitable for a judgement on species status to be made on mating behaviour? Though more research into bedbug fertilization systems would be illuminating, more clarity in the Recognition Concept of species is also necessary before a decision can be made according to this definition.

The plague of bedbugs in northern KwaZulu appears to have, at least partially, been created as a result of DDT-spraying for the control of malaria vectors. Although the treatment of huts with fenitrothion appears to reliably achieve eradication, the possibility exists that both bedbug species will in time develop resistance to organophosphorus compounds, as has happened in other countries in the case of C. lectularius The control of bedbugs by the improvement of standards of hygiene in the rural population is most unlikely under third world conditions, so insecticidal control will possibly remain important for the foreseable future. There is a need for another insecticide to be found

which persists on mud walls long enough to eradicate a bedbug infestation, and to replace fenitrothion should resistance arise.

In the present work, the connection between DDT-spraying and the bedbug nuisance, related on an anecdotal level by Rafatjah (1971), has been demonstrated scientifically. It has also been shown that bedbug control can reduce the antipathy of rural people to the DDT-spraying of their huts for malaria control. However, solving the bedbug problem increases the total cost of malaria control, which emphasises the need for more effective control methods for malaria, perhaps involving vaccination, which in turn would not depend on such a controversial and vulnerable exercise as the intradomiciliary spraying of DDT on an annual basis. Work is definitely needed on the biological control of bedbugs, for at present all our information on this topic is at the anecdotal level.

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