THE SPECIES COMPOSITION AND BIO-ECOLOGY OF *CULICOIDES* SPP. FREQUENTING LIVESTOCK IN THE CENTRAL FREE STATE, SOUTH AFRICA.

by

J.E. LIEBENBERG

Thesis submitted in fulfilment of the requirements for the degree of

Magister Scientiae

In the Faculty of Natural and Agricultural Sciences,

Department of Zoology and Entomology (Entomology Division),

University of the Free State,

Bloemfontein

SUPERVISOR: Prof. T.C. van der Linde

CO-SUPERVISOR: Dr. G.J. Venter

July 2012

Declaration

With the exception of the assistance that has been reported in the acknowledgements and in the appropriate places in the text, this dissertation represents the original work of the author.

No part of this dissertation has been presented for any other degree at any other University.

Candidate…… **Date…….……..……**

ACKNOWLEDGEMENTS

My sincere thanks are due to the following people:

Prof. T.C. van der Linde and Dr. G.J. Venter, my promoters for their guidance and assistance during the project.

The farmers, horse owners and friends who participated and made their facilities available during the survey.

My wife and parents who always supported and encouraged me throughout my career.

The department of Zoology & Entomology, University of the Free State, for the use of facilities and support received.

Ms. Truuske for her time proofing my grammar and spelling.

TABLE OF CONTENTS

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Importance of *Culicoides* biting midges

Culicoides midges (Diptera: Ceratopogonidae) are small, mosquito-like insects that can be a severe biting nuisance to humans in certain parts of the world. They can also cause an acute allergic dermatitis (sweet-itch) in horses, and are the vectors of various pathogens causing economically important diseases of livestock worldwide. Despite their small size, ranging from 1 to 3 mm, members of this genus are associated with the transmission of several different viruses (n = 66), protozoa (n = 15) and filarial nematodes (n = 26) to a diversity of hosts (Meiswinkel *et al*., 2004; Borkent, 2005). At least three orbiviruses, African horse sickness (AHSV), bluetongue (BTV) and epizootic haemorrhagic disease virus (EHDV), cause viral diseases of such international significance that they have been classified as notifiable diseases to the Office International des Epizooties (OIE). In 2005 a fourth virus on the OIE list, vesicular stomatitis virus, was shown to be transmitted by *Culicoides sonorensis* in the United States (Drolet *et al.*, 2005).

The economic impact of these *Culicoides*-borne diseases far outweighs the loss of animals due to viral disease. The costs of vaccination, methods of protecting animals from attack by midges and the production losses in animals have a great impact on the economy. More importantly the presence of especially African horse sickness (AHS) causes economic losses to the country as a result of banning horse exports from areas considered endemic to AHSV (Bosman *et al.*, 1995). This prevents South African horses from participating in international horse events, *e.g.* the Olympic Games and international horse racing events. This can have a great impact on the South African economy as the horse industry is considered to be one of great economic importance to the country.

1.2 General characteristics of *Culicoides* biting midges

Taxonomic identification of *Culicoides* species is based on morphological parameters such as wing pattern and venation, placement and number of sensillae on flagellar segments, intraocular space (between the eyes), form and number of spermatheca and male and female genitalia (Meiswinkel *et al.*, 2004; Borkent, 2005). Molecular

differentiation, using PCR, has been used successfully to differentiate between closely related species (Sebastiani *et al.,* 2001).

Most *Culicoides* species are normally active from just before dusk until just after dawn. While both male and female midges feed on nectar and plant sap, female midges must take a bloodmeal in order to complete their gonotrophic cycle. These blood-seeking females can attack mammals including humans, as well as birds and reptiles (Borkent, 2005). *Culicoides* midges are believed to be exophilic and exophagic (Meiswinkel *et al*., 2000; 2004; 2008). During day time the adult midges mainly rest among bushes, but some species have been found in cracks in tree trunks and in the upper layer of sand (Kettle, 1995).

1.3 Biology of *Culicoides* midges

1.3.1 Geographical Distribution

1.3.1.1 Worldwide distribution

Culicoides midges are found all around the world, with only a few exceptions, notable are the Hawaiian Islands, New Zealand and the most southern tip of South America (Meiswinkel *et al*., 2004). Some selected species are, however, more dominant as vectors in specific zoogeographical areas of the world. The most important and abundant *Culicoides* vectors of orbiviruses include *Culicoides* (*Avaritia*) *imicola* Kieffer in Africa, *Culicoides* (*Monoculicoides*) *sonorensis* Wirth & Jones in North America, *Culicoides* (*Hoffmania*) *insignis* Lutz in South and Central America, *Culicoides* (*Avaritia*) *wadai* Kitaoka, *Culicoides* (*Avaritia*) *brevitarsis* Kieffer, *Culicoides* (*Avaritia*) *actoni* Smith in Australia, *Culicoides* (*Avaritia*) *fulvus* Sen & Das Gupta, *Culicoides* (*Remmia*) *schultzei* Enderlein in Asia, *C. imicola, Culicoides* (*Culicoides*) *pulicaris* L. and *Culicoides* (*Avaritia*) *obsoletus* Meigen in Europe (Mellor, 2004; Tabachnik, 2004).

Culicoides imicola is one of the most widespread species in the word. It can be found throughout Africa, most of the European countries around the Mediterranean Sea, Sri Lanka, Thailand, Laos and Vietnam (Meiswinkel, 1989; Meiswinkel *et al*., 2004; Mellor *et al.,* 2009).

1.3.1.2 South Africa

In South Africa, more than 120 of the 1 400 species known worldwide have been identified (Meiswinkel *et al*., 2004). Very little has, however, been published on the geographical distribution of the *Culicoides* species in South Africa (Venter *et al.*, 1996; Baylis *et al*., 1998).

Based on light trap collections made near livestock *C. imicola* is considered to be the most widespread and abundant livestock-associated species in South Africa. They can become super-abundant and up to 1 million can be collected in one night in a single light trap placed near livestock (Meiswinkel *et al*., 2004).

Culicoides imicola are, however, relatively uncommon in warm/dry and cool/wet areas of South Africa (Jupp *et al.*, 1980; Venter & Meiswinkel, 1994; Venter *et al.*, 1996; Meiswinkel, 1997). The most abundant species in the latter areas were found to be members of the *C. schultzei* group and *Culicoides* (*Hoffmania*) *zuluensis* de Meillon (Venter *et al.*, 1996). *Culicoides imicola* was found to be absent in light trap collections made in the sheep-farming area in the dry Karoo region of South Africa (Jupp *et al.* 1980). This species is also uncommon in the colder high-lying areas of South Africa where *Culicoides* (*Avaritia*) *bolitinos* Meiswinkel was found to be the most abundant (Venter & Meiswinkel, 1994). *Culicoides bolitinos* was shown to be abundant in the winter-rainfall region of the Western Cape Province (Venter *et al.*, 1996, 1997; Nevill *et al.*, 1988), and to be the dominant species, in the absence of *C. imicola*, in the sandy dunefields adjoining Port Elizabeth in the Eastern Cape Province (Meiswinkel, 1997). These areas are considered as endemic for BT and this suggests that other livestock-associated *Culicoides* may play a role in the epidemiology of this particular disease.

1.3.2 Seasonal Distribution

Light trap collections made in South Africa over the last 30 years have shown that *Culicoides* midges are more abundant in areas that seldom experience temperatures below 0°C (Venter *et al*., 1997). These include summer rainfall areas of Limpopo and KwaZulu-Natal Provinces, and the winter rainfall regions of the Southern parts of the Western Cape Province, all of which are below 500 m in altitude (Venter *et al*., 1997; 2006). As the altitude increases, the winter temperatures drop and *Culicoides*

disappear from light trap collections in areas where minimum temperatures decrease below 0°C for more than 30 days. This is then followed by a slow build up during the months following the winter, to a population peak during the favourable conditions in summer (Venter *et al*., 1997; Meiswinkel *et al*., 2004).

Although temperature plays an important role in the maintenance of *Culicoides* populations, rainfall is just as important in maintaining breeding sites (Meiswinkel *et al*., 2004). During the summer months, *Culicoides* numbers will increase gradually as long as there is sufficient rainfall to sustain the semi-aquatic habitats for the larval stages. Irrigation can play an important role in sustaining high populations (Meiswinkel *et al*., 2004).

1.3.3 Life Cycle

All *Culicoides* species display a typical holometabolous life-cycle consisting of eggs, four larval instars, pupa and the adult midges.

The eggs are small and slender, resembling a sausage in shape, measuring 350-500 µm in length and 65-80 µm in breadth (Kettle, 1995; Day *et al*., 1997). Eggs tend to be white when laid but turn dark brown to black after a short time (Borkent, 2005). Egg batches can vary from 30-40 in the Australian *C. brevitarsis* up to 450 in *Culicoides circumscriptus* found in Britain and most of Europe to Russia, and from North Africa to Israel (Kettle, 1995). In *C. imicola* the batch size varies from 53 to 69 (Nevill, 1967; Braverman & Linley, 1994). In most species the eggs hatch within a few days in favourable temperatures, but they can also enter into diapause and will then not hatch for 7-8 months (Kettle, 1995).

Culicoides larvae are typical nematocerean with a well sclerotized head, 11 body segments and no appendages (Kettle, 1995, Nevill, 1967; 1969). In most cases the larval stages are of much longer duration than the egg and pupal stages (Kettle, 1995). Larval development is temperature-dependent, ranging from 11–16 days at 28 °C, 15–21 days at 25 °C to as long as 34–56 days at 20 °C in *C. imicola* (Veronesi *et al*., 2009). Under field conditions, life stages of different *Culicoides* species may vary from as short as two weeks in the dung-breeding *C. brevitarsis*, up to nearly a year in some arctic species (Kettle, 1995). The larvae of some species are carnivorous and

feed on protozoa, rotifers and nematodes (Linley, 1979). The fourth stage larvae of some species may even cannibalise second stage larvae (Nevill, 1967; 1969).

The pupa is a short lived, non-feeding stage which gives rise to the winged adult (Kettle, 1995). Contrary to most other *Culicoides* species *C. imicola* pupae are unable to float in water and drown in water-logged soil (Nevill, 1967). The gradual drying of larval habitats will promote pupation.

Similar to mosquitoes, *Culicoides* males also commonly emerge before the females (Kettle, 1995). This leads to a population of males in the breeding area, ready to mate when the females emerge. In most species, like *C. brevitarsis*, mating occurs during swarming just before sunset. Swarm size, consisting mainly of males, may vary from 10 to 1 000 individuals, but is more commonly around 50 in *C. brevitarsis* (Kettle, 1995).

The adult midge life span also varies depending on ambient conditions, but they usually survive less than 20 days, although they may occasionally live for up to 63 to 90 days (Nevill, 1971; Mellor *et al*., 2000). Females usually require one blood meal for each batch of eggs matured. Therefore, the frequency of blood feeding is linked to the rate of egg development, which is linked to the species and ambient temperature (Mullens *et al*., 2004). They usually only fly short distances from their larval habitat (Kettle, 1995), but can be carried on the wind for distances of possibly up to 700 km (Sellers *et al*., 1977; Sellers, 1992).

1.3.4 Immature habitats

Although the basic requirements for larval habitats are moisture and a medium containing organic matter most species have very specialized larval habitats (Dyce & Marshall, 1989; Meiswinkel, 1989; Meiswinkel & Linton, 2003; Meiswinkel *et al*., 2004; Nevill *et al.*, 2007; 2009).

There are basically four main types of *Culicoides* larval habitat (Meiswinkel *et al*., 2004):

(i) Surface water and a soil interface. About half the known *Culicoides* species in southern Africa make use of some combination of soil and water to lay their

eggs (Blanton & Wirth, 1978; Meiswinkel *et al*., 2004). The soil may vary from coarse sand to the finest of clay, often enriched with some type of decomposed plant material (Meiswinkel *et al*., 2004). The water may vary from fresh flowing streams to polluted stagnant pools with varying degrees of acidity, salinity and alkalinity (Meiswinkel *et al*., 2004). In the Onderstepoort area *C. imicola* have been found to breed in wet, organically enriched kikuyu (*Pennisetum clandestinum*) pastures, and that these breeding sites can expand during years of exceptional rainfall (Meiswinkel *et al*., 2004).

(ii) Dung pats of large mammals. In 1968 it was found that *Culicoides* midges can breed in bovine dung (Nevill, 1968). There are at least ten *Culicoides* species, all of the subgenus *Avarita*, that require the fresh dung of certain animals to complete their life cycles (Dyce & Marshall, 1989). *Culicoides bolitinos* is known to breed in the dung of cattle, African buffalo (*Syncerus caffer*) and sometimes blue wildebeest (*Connochaetes taurinus*) (Meiswinkel, 1989; Meiswinkel *et al*., 2004). Other species are known to breed in the dung of zebra (*Equus burchelli*), elephant (*Loxodonta africana*) and black and white rhinoceros (*Ceratotherium simum*; *Diceros bicornis*) (Meiswinkel *et al*., 2004; Nevill *et al*., 2007; 2009).

(iii) Tree holes, plant and rock cavities. These larval habitats vary from deep, dark, water-filled holes to shallow, exposed but moist hollows in trees (Blanton & Wirth, 1978; Meiswinkel *et al*., 2004). About 15% of *Culicoides* in southern Africa are known or suspected to breed in these habitats and it is assumed that they feed on birds for their primary source of blood (Meiswinkel *et al*., 2004).

(iv) Rotting fruits and plants. These larval habitats have yet to be investigated thoroughly, but some *Culicoides* species have been found in the rotting stems of the banana plant and rotting fallen fruit (Blanton & Wirth, 1978), *e.g. Culicoides tuttifrutti* has been reared from the rotting fallen fruits of the sausage tree (*Kigelia africana*) and the maroela tree (*Sclerocarya caffra*) (Meiswinkel & Linton 2003; Meiswinkel *et al*., 2004).

1.3.5 Hosts preference

Host preference will influence the biting rate of a species and is therefore one of the critical factors that will influence the vector capacity of a *Culicoides* species (Mullens *et al.,* 2004). The trapping of large numbers of certain *Culicoides* near specific host animals is generally used as an indicator of host preference (Meiswinkel *et al*., 2004). Recent studies have, however, shown that the number of *Culicoides* species collected with light traps is not necessarily comparable to species diversity and host bite rate (Carpenter *et al*., 2008c; Gerry *et al*., 2008; 2009).

Blood meal identification of freshly engorged *Culicoides* females has shown that at least 13 species will feed on horses (Meiswinkel *et al.,* 2004). Similarly it was shown that at least 12 or 13 species will feed on cattle and sheep respectively (Meiswinkel *et al.,* 2004). Most of these will, however, feed on any of these larger mammals. While some species have shown a tendency to feed on large mammals others prefer avian hosts (Meiswinkel *et al*., 2004; Borkent, 2005).

1.4 *Culicoides* midges as vectors of disease

Due to their blood-feeding habits *Culicoides* midges are associated with a number of diseases and micro-organisms but it is as vectors of viruses that they are of the greatest veterinary importance. *Culicoides* midges were proven to be the vectors of the BTV in 1944 by Du Toit (1944) and to date more than 75 arboviruses, belonging mostly to *Bunyaviridae*, *Reoviridae* and *Rabdoviridae* families, have been isolated from different *Culicoides* species worldwide (Meiswinkel *et al*., 2004; Borkent, 2005).

1.4.1 Vector capacity and vector competence

Many factors can influence the intricate three-way relationship that exists between virus, vector and the vertebrate host and the successful transmission of an arbovirus to a susceptible host (Hardy *et al.,* 1983). The vectorial capacity of a *Culicoides* species refers to its ability to successfully transmit a pathogen. It can be defined as the average number of infective bites delivered by a *Culicoides* midge feeding on a single host in one day and is a combination of midge density in relation to the animal, host

preference, midge biting frequency, life-span of infected midge, duration of viremia and vector competence (Dye, 1992; Mullens *et al*., 2004).

Vector competence which refers to the ability of the vector to support infection, replication, dissemination and transmission of the virus is one of the critical factors determining vector capacity. Although vector competence is under genetic control in the insect vector (Tabachnick, 1991) it can be greatly influenced by environmental factors (Wellby *et al.,* 1996; Mellor *et al.*, 1998; Wittmann *et al.,* 2001).

After ingestion by a *Culicoides* vector, most arboviruses replicate in the cells of the mesenteron, then penetrate the basal lamina and are released into the haemolymph to set up cycles of infection and replication (Mellor *et al.,* 2000). A number of barriers, inside the vector, inhibit arbovirus infection, most notably a transovarial transmission barrier (Mellor *et al.,* 2000). Therefore only parous females that have had a blood meal and completed a gonotrophic cycle can transmit a virus (Nelson & Scrivani, 1972; Nunamaker *et al.,* 1990). The ratio of parous to nulliparous females, or those that have not completed a gonotrophic cycle, can therefore give an indication of the vector potential of a *Culicoides* population (Venter *et al*., 1997).

Arboviruses must first infect and replicate in the salivary glands before they can be transmitted during subsequent feedings on the next susceptible host. This period is also dependent on temperature and can take from one to two weeks (Paweska *et al*., 2002).

It should be emphasised that neither laboratory demonstration of vector competence (Mullens *et al*., 2004) nor the isolation of a virus from a field-collected insect is sufficient proof of a species to be a proven vector of a specific virus (Walton, 2004).

A vector with low competence may be more efficient at virus transmission than a competent vector with low vector capacity due to low biting rates or survivorship. For example, in Australia *C. brevitarsis* has a low competence for BTV, but effectively transmits the virus due to its high biting rate, while *C. fulvus* which is more competent, has a lower vectorial capacity due to lower abundance and limited geographical distribution (Standfast *et al.,* 1972).

Based on their abundance near livestock the *Culicoides* species probably having the highest potential as orbivirus vectors in South Africa are *C. imicola*, *C. bolitinos*, *Culicoides gulbenkiani*, some members of the *C. shultzei* group, *C. zuluensis*, *Culicoides magnus* and *Culicoides pycnostictus* (Nevill *et al.*, 1992b; Venter *et al*., 1996; Meiswinkel *et al*., 2004).

In South-Africa BT and AHS are the most important notifiable diseases spread by *Culicoides* midges. They occur annually in the northern and eastern parts of South Africa and cause severe disease in sheep and horses respectively. Most cases of AHS and BT occur late in the summer, from March to May, coinciding with the greatest number of *Culicoides* in light trap collections (Venter *et al*., 1996; 1997).

1.4.2 *Culicoides* as vectors of African horse sickness virus (AHSV)

AHS is not contagious, and the disease is spread to other areas by movement of infected animals or vectors (Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004; Maclachlan & Guthrie, 2010). The causative agent AHSV is a double-stranded RNA virus, within the genus *Orbivirus* of the family *Reoviridae*, which causes an infectious, non-contagious, disease of equids (McIntosh, 1958; Howell, 1962). The virus exists as nine distinct serotypes (McIntosh, 1958; Howell, 1962), all of which are endemic in sub-Saharan Africa.

AHS has been known in Africa for many centuries, and was first noticed in South Africa after the introduction of horses from Europe, more than 300 years ago (Bosman *et al.,* 1995; Coetzer & Guthrie, 2004). AHS is the most lethal infectious disease, with up to 95% mortalities in susceptible equines (Baylis *et al.*, 1999; Coetzer & Guthrie, 2004; Maclachlan & Guthrie, 2010). Donkeys and mules seem to be less susceptible and generally develop milder symptoms (Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004; Maclachlan & Guthrie, 2010).

The outcome of infection, including the incubation period and severity of disease, depends on the form of AHS and the susceptibility of the host. The pulmonary form, also known as "Dunkop", occurs most commonly when AHSV infects fully susceptible horses (Coetzer & Guthrie, 2004). Following the incubation period, a fever may be the only sign for a day or two, with temperatures of 41°C or even higher (Coetzer & Guthrie, 2004). The characteristic symptoms of this form are severe

dyspnoea, paroxysms of coughing and discharge of large quantities of frothy, serofibrinous fluid from the nostrils (Coetzer & Guthrie, 2004). Less than 5% survive the pulmonary form of AHS (Coetzer & Guthrie, 2004).

The cardiac form, also known as "Dikkop", is characterized by swelling of the head and neck, and particularly the supraorbital fossae (Coetzer & Guthrie, 2004). Some animals only develop a mild fever (Coetzer & Guthrie, 2004). Varying degrees of swelling of the supraorbital fossae and other parts of the head are evident and in horses, bulging of the supraorbital fossae is characteristic. In severe cases the eyelids, lips, cheeks, tongue, intermandibular space and sometimes also the neck, chest and shoulders are involved but generally not the lower parts of the legs (Coetzer & Guthrie, 2004). Some animals may repeatedly lie down or are restless when standing, and frequently paw the ground with their front feet as a result of severe colic (Coetzer & Guthrie, 2004). The cardiac form is always more protracted than the pulmonary, with a mortality rate of about 50% (Coetzer & Guthrie, 2004). The most common is the mixed form, but it is very rarely diagnosed as such and symptoms of both types can occur in a variety of sequences and the mortality rate is approximately 70% (Coetzer & Guthrie, 2004).

Mortalities due to AHS occur in South Africa every year, Major epizootics occur every 10 to 15 years (Baylis *et al.,* 1999). The most severe outbreak of AHS to date was in 1855, when nearly 70 000 horses died in the Western Cape Province (Bayley, 1856). Since the polyvalent AHS vaccine became available in southern Africa, severe losses have largely ceased, although they continue to occur in individual or small groups of horses (Coetzer & Guthrie, 2004). Outbreaks of AHS in 1999 and 2004 in the surveillance zone of the AHS-free area in Stellenbosh had considerable financial implications and on both occasions led to a two-year embargo on the export of horses from South Africa (Bosman *et al.,* 1995). Outbreaks outside the endemic areas of the disease have served as a warning that the disease may spread to countries that have been free of AHS up to now (Howel, 1960; Mellor, 1993; Coetzer & Guthrie, 2004). AHS occurs regularly in sub-Saharan Africa, and is endemic to eastern and central Africa (Coetzer & Guthrie, 2004; Maclachlan & Guthrie, 2010). From there the disease spreads down through South Africa (Coetzer & Guthrie, 2004).

In South Africa the disease was believed to extend from the northern lowveld, southwards, depending on the midge numbers which in turn are influenced by the climatic conditions and availability of breeding sites (Guthrie, 1999; Coetzer & Guthrie, 2004). AHS occurs in the northern parts of Mpumalanga and KwaZulu Natal annually during the summer and in recent years, relatively large outbreaks of AHS have occurred approximately every five to ten years in the Free State. In the summer rainfall areas, AHS is mostly prevalent in warm coastal regions or low-lying, moist inland areas such as valleys, marshes and in riverine vegetation during the second half of the summer (Coetzer & Guthrie, 2004). Early heavy rains followed by warm, dry spells favour the occurrence of epidemics. In South Africa, the first cases of AHS usually occur at the beginning of February, but the most serious outbreaks commonly occur in March and April (Venter *et al*., 1997; Coetzer & Guthrie, 2004). Midge numbers decrease rapidly and outbreaks disappear abruptly following the first frost, usually during late April or May, however, in areas with less frost, deaths may occur in May and even June (Coetzer & Guthrie, 2004). In non-endemic areas, outbreaks do not continue where they stopped the year before, apparently the disease re-emerges from the northern parts every year (Guthrie, 1999; Coetzer & Guthrie, 2004). In fact, 120 000 to 150 000 doses of AHS vaccine that are issued annually by Onderstepoort Biological Products (OBP), are used in the more northerly regions of South Africa.

During outbreaks of AHS in endemic areas, different serotypes may be active simultaneously, but usually one dominates during a particular season (Coetzer & Guthrie, 2004). Serotypes 1 to 8 are all highly pathogenic and cause 90 to 95% mortality while serotype 9 is slightly less pathogenic and results in mortality of about 70% (Coetzer & Guthrie, 2004). In North and West Africa there are areas where horses have been present since at least 2 000 BC, and they have apparently acquired a natural resistance (Coetzer & Guthrie, 2004). In addition to equines, dogs are the only other animals to contract the disease after eating infected horse meat (Coetzer & Guthrie, 2004; Maclachlan & Guthrie, 2010).

The decline in AHS outbreaks in the south over the last few decades was ascribed to the elimination of large free-ranging populations of zebra (cycling host) and the introduction of a polyvalent AHS vaccine in 1974 which created a barrier of immune horses.

The possibility that AHSV could be transmitted by small biting insects was suggested by Pichford and Theiler in 1903 (Coetzer & Guthrie, 2004). Cumulative oral susceptibility studies over the past 10-15 years in South Africa indicate that at least 13 South African *Culicoides* species, belonging to some eight subgenera, are potentially involved in the epidemiology of AHSV. The subgenera and *Culicoides* species from which virus could be isolated 10 days after feeding on a virus-infected blood meal are: *Avaritia* (*C. imicola*, *C. bolitinos*, *C. gulbenkiani*); *Hoffmania* (*C. zuluensis*); *Monoculicoides* (*Culicoides expectator*); *Culicoides* (*C. magnus, Culicoides brucei)*; *Remmia* (*Culicoides enderleni*); *Meijerehelea* (*Culicoides leucostictus*); *Beltranmyia* (*C. pycnostictus*), *Pontoculicoides (Culicoides engubandei)*; *Synhelea* (*Culicoides dutoiti*) and one *Culicoides* species (*Culicoides bedfordi*) not allocated to a specific subgenus (Paweska *et al.,* 2003; Venter *et al*., 2003; Venter & Paweska, 2007; Venter *et al.,* 2009). These susceptibility results are supported by field isolations of AHSV from *C. imicola* and *C. bolitinos* (Nevill *et al.,* 1992a; Venter *et al.,* 2006).

1.4.3 *Culicoides* as vectors of bluetongue virus (BTV)

Bluetongue (BT) is an arthropod-borne viral disease of domestic and wild ruminants, especially sheep (Verwoerd & Erasmus, 2004). The causative agent, BTV, is a double-stranded RNA virus, within the genus *Orbivirus* of the family *Reoviridae* (Borden *et al.* 1971). Neitz (1948) confirmed that there are multiple serotypes of the BTV and this provided an explanation for the vaccination failures that had been experienced. BTV exists as a number of serotypes of which 24 have been identified to date (Howell, 1969).

The disease is characterized by symptoms such as inflammation, haemorrhage, ulceration and cyanosis of the mucus membranes of the oronasal cavity, coronitis, laminitis, oedema of the head and neck and torticollis (Verwoerd & Erasmus, 2004; Maclachlan & Guthrie, 2010). Foetal abnormalities may occur if the ewe becomes infected early during pregnancy (Verwoerd & Erasmus, 2004; Maclachlan & Guthrie, 2010). The disease was first recognized when Merino sheep were introduced from Europe into the Western Cape Province in the late eighteenth century, even then the disease was known to be prevalent throughout the summer months (Verwoerd & Erasmus, 2004). The course of the disease in sheep can vary from peracute to a chronic form with mortalities between 2 and 30% (Verwoerd & Erasmus, 2004). The

peracute form is usually more aggressive and the animal can die within seven to nine days of infection, mainly as a result of lung oedema and eventual asphyxia (Verwoerd & Erasmus, 2004). In chronic cases death can result from secondary bacterial pneumonia and exhaustion, or recovery can be prolonged while mild cases usually recover rapidly and completely (Verwoerd & Erasmus, 2004).

Until 1995 the worldwide distribution of BTV, on almost all continents, lay approximately between latitudes 40°N and 35°S (Mellor & Boorman, 1995; Tabachnick, 2004; Maclachlan & Guthrie, 2010). Since 1998, however, the virus has greatly expanded its distribution and outbreaks have occurred over 800 km further north, up to latitude 44°30'N, than previously recorded (Purse *et al.*, 2005). During the last decade at least five serotypes of BTV (1, 2, 4, 9 and 16) became endemic in southern Europe (Saegerman *et al*., 2008). During 2006 outbreaks of BT continued in southern Europe and in August 2006 a sixth BTV serotype (BTV-8) caused a severe outbreak of BT among sheep and cattle in northern Europe. During that year BT was recorded from the Netherlands, Belgium, Germany, Luxembourg and the north of France up to a 52ºN (Elbers *et al*., 2006). The virus overwintered (2006-2007) in northern Europe and reappeared in affected areas during May-June 2007, then spread further into Germany and France, reaching Denmark, Switzerland, the Czech Republic and the United Kingdom (Schwartz-Cornil *et al.*, 2008).

Virus distribution is dependent on the availability of reservoir and amplifying hosts such as game and cattle and on suitable *Culicoides* species in adequate numbers to transmit the virus between host animals (Verwoerd & Erasmus, 2004). Therefore the distribution of BT closely resembles that of the *Culicoides* vector species, temporally and spatially (Tabachnick, 2004; Verwoerd & Erasmus, 2004).

BTV may overwinter in cattle and a viral release mechanism may be triggered by bites of the vector midge (Takamatshu *et al*., 2003). The build-up in midge numbers especially parous females towards the end of summer increases the chances for the transmission of virus, and therefore the occurrence of disease (Venter *et al.*, 1996).

The first evidence that proved *Culicoides imicola* to be a vector of BT was obtained by Du Toit (1944). Afterwards *Culicoides sonorensis* (= *variipennis*) and other *Culicoides* species were also proven to be vectors in the USA and Australia

(Verwoerd & Erasmus, 2004). BTV has been isolated from various species of *Culicoides* from around the world, *C. imicola* being the most important in Africa and the Middle East, *C. variipennis* and *C. insignis* in North America and *C. fulvus* in Australia (Meiswinkel *et al.*, 2004; Verwoerd & Erasmus, 2004). BT is not contagious and very little virus is present in the excretions of infected animals (Verwoerd & Erasmus, 2004).

Culicoides imicola is the only proven vector of the BTV in South Africa (Meiswinkel, 1989), but *C. bolitinos* that has a close association with cattle, even breeding in bovine dung, is strongly suspected to be a vector as bovines are a reservoir and amplifying host of the BTV (Venter *et al.*, 1996). This is supported by the occurrence of BT in the colder high lying areas of central South Africa, where *C. imicola* is rare and species like *C. bolitinos*, *C. zuluensis* and *C. pycnostictus* are the dominant species (Venter *et al*., 1998).

Cumulative oral susceptibility studies over the last 10-15 years in South Africa indicate that at least 13 *Culicoides* species, belonging to some eight subgenera, are potentially involved in the epidemiology of BTV. The subgenera and *Culicoides* species from which virus could be isolated 10 days after having fed on a virus-infected blood meal are: *Avaritia* (*C. imicola*, *C. bolitinos*, *C. gulbenkiani*); *Hoffmania* (*C. zuluensis*, *Culicoides milnei*); *Monoculicoides* (*Culicoides huambensis*, *C. expectator*); *Culicoides* (*C. magnus*); *Remmia* (*C. enderleni*); *Meijerehelea* (*C. leucostictus*); *Beltranmyia* (*C. pycnostictus*), and two *Culicoides* species (*C. bedfordi*, *Culicoides angolensis*) not allocated to a specific subgenus (Venter *et al*., 1998, 2004, 2006; Venter & Paweska, 2007; Paweska *et al.*, 2002). These susceptibility results are supported by field isolations of BTV from at least five of these South African livestock-associated species, *C. imicola*, *C. bolitinos*, *C. milnei*, *C. pycnostictus* and *C. expectator* (Walker & Davies, 1971; Nevill *et al*., 1992b; Barnard, 1998).

The potential for other *Culicoides* species to be involved in the epidemiology of BTV in South Africa is highlighted by the fact that both *C. imicola* and *C. bolitinos* were absent from light trap collections made in the sheep-farming area in the Karoo region (Jupp *et al*., 1980), which is considered to be endemic for BT. These results indicate that susceptibility to BTV may indeed be widespread in the genus *Culicoides.* The outbreaks of BTV in northern Europe in the absence of *C. imicola* (Thiry *et al.*, 2006;

Meiswinkel *et al*., 2007; Mellor *et al*., 2009), highlighted the notion that more than one *Culicoides* species might be involved in the epidemiology of this and other orbiviral diseases transmitted by *Culicoides* midges (Mellor & Pitzolis, 1979; Mellor, 1992; Carpenter *et al*., 2009). Limited susceptibility data (Goffredo *et al*., 2004; Carpenter *et al*., 2006; 2008b) seem to indicate that the susceptibility and vector competences of some of the Palaearctic *Culicoides* species could be equal to or even higher than that of the proven vector *C. imicola*. The potential involvement of a variety of *Culicoides* species, each with a unique and mostly unstudied biology, greatly increases the complexity of the epidemiology of this virus.

1.5 Control of *Culicoides*-spread disease

Vector-borne viral disease has three focal points for control, the vector, the virus and the target animal, and when possible these actions should be integrated into a control system. Integrated control methodologies comprise of chemical, biological and environmental procedures used jointly or sequentially against a background of an exhaustive ecological understanding of the selected target pest or vector, so as to maximise efficacy, and be fully acceptable from the health and environmental standpoint. In Africa with its variety of possible reservoir hosts, eradication of the disease is impossible, but immunisation of target animals should limit the incidence of disease (Meiswinkel *et al.,* 2004). At present vaccination is still the most efficient and reliable way to minimise the impact of these diseases.

Control of *Culicoides* species has been met with limited success (Cilek & Kline, 2002; Carpenter *et al.,* 2008a), because of the wide variety of immature habitats, small size, and large numbers of the midges. Eradication of *Culicoides* is impossible because adults occur in such large numbers and they have such widespread and diverse larval habitats (Meiswinkel *et al*., 2004). Altering of larval habitats in less favourable areas however may reduce the adult numbers (Holbrook, 1982).

Chemical control of *Culicoides* midges has its advantages, but evaluation of these compounds is difficult as a result of differences in susceptibility of midge species and also that of laboratory colonies to field populations (Carpenter *et al*., 2008a). It has also been demonstrated that *Culicoides* midges can transmit viruses successfully before being incapacitated by certain chemical compounds (Mullens *et al*., 2000). The

use of repellents to decrease biting rates on livestock may form an essential part of an integrated control system. In Europe some compounds have been shown to reduce the biting rates of *Culicoides* in humans (Trigg, 1996; Carpenter *et al.*, 2005). In South Africa studies have shown that treating a polyester mesh with different compounds has a significant repellent effect against *Culicoides* midges (Page *et al*., 2009; Venter *et al*., 2011).

Susceptible animals are stabled from dusk till dawn since that is the most active period for the midges and they are believed not to enter stables (Meiswinkel *et al*., 2004). Whilst stabling is recommended for control it has been shown that *Culicoides* do enter stables, and horses are protected from *Culicoides* bites only if the stables are adequately closed (Barnard, 1997; Meiswinkel *et al*., 2000). Closing stables by meshing with synthetic gauze resulted in a 14-fold reduction in the numbers of *Culicoides* entering (Meiswinkel *et al*., 2000). Animals should also be kept away from the warmer low-lying areas during periods of high risk for transmission of viral diseases (Meiswinkel *et al*., 2004).

Apart from supportive treatment, there is no specific therapy for AHS (Coetzer & Guthrie, 2004). All serotypes of AHSV are distributed throughout South Africa and the use of a polyvalent vaccine is therefore necessary to protect horses (Coetzer & Guthrie, 2004). Until 1990 the attenuated live-virus vaccine comprised of two quadrivalent vaccines, one containing serotypes -1, -3, -4 and -5 and the other serotypes -2, -6, -7 and -8. Due to safety problems, the vaccine strain of AHSV-5 was discontinued in 1990 (Van Dijk, 1998).

In the case of an outbreak in a disease-free area, attempts should be made to limit further transmission and to eradicate it as soon as possible. According to Coetzer and Guthrie (2004) the following measures should be taken at outbreaks in epidemic situations: (i) delineate the area of infection; (ii) prevent the movement of all equine animals within, into and out of the infected area; (iii) stable equine animals from dusk till dawn and institute insect control methods; (iv) monitor the animals' temperatures twice daily to detect infection as soon as possible; (v) vaccinate all susceptible animals immediately with the polyvalent vaccine; and (vi) identify the vaccinated animals. Under outbreak situations, however, the use of live attenuated vaccines may not be appropriate (Mellor & Hamblin, 2004) so it would therefore be essential to

determine the serotype responsible for the outbreak and administer a monovalent vaccine. It must be kept in mind that AHS is a notifiable disease and all suspected cases need to be reported to a State veterinarian who must notify the OIE immediately.

Control of a vector-borne disease like BT varies according to whether outbreaks are in endemic regions or areas that are usually disease free (Verwoerd & Erasmus, 2004). In endemic areas management of the disease by limiting the occurrence and economic impact is usually the norm, whereas eradication is the preferred method of control in areas usually free of disease (Verwoerd & Erasmus, 2004). The outbreak of BT in Europe and subsequent overwintering of the virus demonstrated that this is not always possible.

1.6 Importance of *Culicoides* in the Free State

The Free State province is situated in the center of South Africa, bordered by the Gauteng province in the north where AHS is relatively abundant (Venter *et al.,* 1999). Bloemfontein in particular is situated on one of the main connecting roads between Gauteng in the north and Western Cape in the south. *Culicoides*-borne diseases occur in the Free State but to a limited degree as a result of the cold winters that limit the development of midges. This implies that an almost new population of midges needs to build up every summer. In spite of climatic limitations there are still periods of high risk for disease at the end of the warm rainy season when the midge population peaks. Sheep farming plays an important role in the economy of the Free State. Horses are important as national shows are held in Bloemfontein annually and a range of other events. There are also some horse breeders that are situated in the Free State.

During the 2010/2011 season, 83 cases of AHSV were reported as opposed to 22 cases reported from 2006 to 2010 (AHS Trust). This indicates not only an increase in the disease incidence, but also an increase in awareness of AHSV as suspected cases are more frequently and intensively being monitored and reported.

Culicoides midges can occur readily and in large numbers in the Free State (Venter *et al*., 1996). The presence of midges in these parts also leads to outbreaks of diseases associated with them. *Culicoides* midges have been collected in the Free State year round, but to date very little is known about the midge species occurring in the Free

State and their seasonality. This lack of information made it important to conduct research and to gather data on the midge populations in this part of South Africa. Midge-borne diseases seem to be increasing in the Free State, adding to the importance of research on *Culicoides* in these parts.

To date several studies have been undertaken to establish the occurrence and importance of midges in South Africa, however, only a few investigated the situation in the Free State. This study will be the first effort of this magnitude to establish the species composition and seasonality of the *Culicoides* population in the central Free State. AHS and BT occur readily in this area annually and there is a real need to determine the population dynamics of *Culicoides* in this area.

1.7 Thesis Plan

Awareness of all potential vectors of orbiviruses will be crucial for the development and implementation of effective integrated control measures and disease risk analysis.

In order to determine the risk of AHS and other arbovirusses occurring in the Free State the species as well as the seasonal patterns of *Culicodes* midges in the central Free State were determined. An effort was made to determine the influence of temperature and rainfall on the occurrence and abundance. The suitability of the light trap as a monitoring tool and factors influencing the efficacy of light traps are discussed. In addition host preference of the midge species occurring in the Bloemfontein area and factors which might influence oviposition preferences and larval habitats of *Culicoides* midges were investigated. As an initial step in this study the efficacy of the newly developed 220V and 12V Free State light traps, used in the present study, were compared to that of the Onderstepoort light trap.

Awareness of all potential vectors of AHSV and the factors that could influence this should contribute to a better understanding of the epidemiology of AHSV and the determination of high risk periods in the Free Sate.

1.8 References

African Horse Sickness Trust 2012 African horse sickness. Online at: http://www. africanhorsesickness.co.za (accessed 2 January 2012).

Barnard, B.J.H. 1997. Some factors governing the entry of *Culicoides* spp. (Diptera: Ceratopogonidae) into stables. *Onderstepoort Journal of Veterinary Research*, **64**, 227–233.

Barnard, B.J.H. 1998. Epidemiology of African horse sickness and the role of the zebra in South Africa. *Archives of Virology (Supplement)*, **14**, 13-19.

Bayley, T.B. 1856. Notes on the horse-sickness at the Cape of Good Hope, in 1854-'55. Cape Town: Saul Solomon & Co., Steam Printing Office, Longmarket Street. p 118.

Baylis, M., Meiswinkel, R. & Venter, G.J. 1998. A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *Journal of the South African Veterinary Association*, **70**, 80-89.

Baylis, M., Melor, P.S. & Meiswinkel, R. 1999. Horse sickness and ENSO in South Africa. *Nature*. **397**, 574.

Blanton, F.S. & Wirth, W.W. 1978. The sand flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry (Gainesville, Fla.) p 204

Borden, E.C., Shope, R.E. & Murphy, F.A. 1971. Physiochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus – a new taxonomic group. Physiochemical and serological studies. *Journal of General Virology*, **13**, 261-271.

Borkent, A. 2005. The biting midges, the Ceratopogonidae (Diptera). In W.C. Marquardt, *Biology of disease vectors*, 2nd ed., Elsevier Academic Press, San Diego, p. 113-126.

Bosman, P., Brückner, G.K. & Faul, A. 1995. African horse sickness surveillance systems and regionalization/zoning: the case of South Africa. *Revue Scientifique et Technique*. **14**, 645-653.

Braverman, Y. & Linley, J.R. 1994. Fecundity and proportions of gravid females in populations of the bluetongue vector *Culicoides imicola* (Diptera: Ceratopogonidae) and several other species in Israel. *Journal of Medical Entomology*, **31**, 838–843.

Carpenter, S., Eyres, K., McEndrick, I., Smith, L., Turner, J., Mordue, W., Mordue, A.J. (Luntz). 2005. Repellent efficiency of BayRepel® against *Culicoides impunctatus* (Diptera: Ceratopogonidae). *Parasitology Research*, **95**, 427–429.

Carpenter, S., Lunt, H.L., Arav, D., Venter, G.J. & Mellor, P.S. 2006. Oral susceptibility to bluetongue virus of *Culicoides* (Diptera: Ceratopogonidae) from the United Kingdom. *Journal of Medical Entomology*, **43**, 73–78.

Carpenter, S., McArthur, C., Selby, R. Ward, R., Nolan, D.V., Mordue Luntz, A. J. Dallas, J. F. Tripet, F. & Mellor, P.S. 2008c. Experimental infection studies of U.K. *Culicoides* species with bluetongue virus serotype 8 and 9. *The Veterinary Record*, **163**, 589–592.

Carpenter, S., Mellor, P.S., Torr, S.J. 2008a. Control techniques for *Culicoides* biting midges and their application in the U.K. and northwestern Palaearctic. *Medical and Veterinary Entomology*, **22**,175–187.

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. & Mellor, P. 2008b. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector? *Journal of applied Ecology*, **45**, 1237-1245.

Carpenter, S., Wilson, A., Mellor, P.S., 2009. *Culicoides* and the emergence of bluetongue virus in northern Europe. *Trends in Microbiology*, **17**, 172-178.

Cilek, J.E. & Kline, D.L. 2002. Adult biting midge response to trap type, carbon dioxide, and an octenol-phenol mixture in northwestern Florida. *Journal of the American Mosquito Control Association*, **18**, 228-231

Coetzer J.A.W. & Guthrie A.J., African horse sickness. In: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, 2004, pp. 1231–1246.

Day, J.F., Duzak, D., Braverman, Y., Chizov-Ginsberg, A. & Linley, J.R. 1997. Ultrastructure of the eggs of *Culicoides circumscriptus, Culicoides gejgelensis,* and *Culicoides imicola* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association,* **13**, 76-83.

Drolet, B.S., Campbell, C.L., Stuart, M.A. & Wilson, W.C. 2005. Vector competence of *Culicoides sonorensis* (Diptera: Ceratopogonidae) for vesicular stomatitis virus. *Journal of Medical Entomology*, **42**, 409-418.

Du Toit, R.M. 1944. The transmission of Blue-Tongue and Horse Sickness by *Culicoides. Onderstepoort Journal of Veterinary Science and Animal Industry*, **19**, 7-16.

Dyce, A.L. & Marshall, B.D. 1989. An early record of *Culicoides* species (Diptera: Ceratopogonidae) developing in the dung of game animals in southern Africa. *Onderstepoort Journal of Veterinary Research*, **56**, 85–86.

Dye, C. 1992. The analysis of parasite transmission by bloodsucking insects. *Annual Review of Entomology*, **37**, 1-19.

Elbers, A.R.W., Backx, A., Meroc, E., Gerbier, G., Staubach, Hendrickx, C.G., Van der Spek, A. & Mintiens, K. 2006. Field observations during the bluetongue serotype 8 epidemic in 2006: I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France and the Netherlands. *Preventive Veterinary Medicine*, **87**, 21-30.

Gerry, A.C., Monteys, V.S.I., Vidal, J-O.M., Francino, O. & Mullens, B.A. 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in Northeastern Spain in relation to midge capture using UV light and Carbon Dioxide-Baited traps. *Journal of Medical Entomology,* **46**, 615-624.

Gerry, A.C., Nawaey, T.M., Sanghrajka, P.B., Wisniewska, J. & Hullinger, P. 2008. Hematophagous diptera collected from a horse and paired carbon dioxide-baited suction trap in southern California: relevance to West Nile virus epizootiology. *Journal of Medical Entomology*, **45**, 115-124

Goffredo, M., Romeo, G., Monaco, F., Di Gennaro, A. & Savini, G. 2004. Laboratory survival and blood-feeding response of wildcaught *Culicoides obsoletus* complex (Diptera: Ceratopogonidae) through natural and artificial membranes. *Veterinaria Italiana*, **40**, 282–285.

Guthrie, A.J. 1999. Regionalisation of South Africa for African horse sickness. In: *Proceedings of the 8th International Conference on Equine Infectious Diseases* (ed. by U. Wernery, J.F. Wade, J.A. Mumford and O.-R. Kaaden), pp. 376-379. 23-26 March 1998, United Arab Emirates, Dubai.

Hardy, J.L., Houk, E.J., Kramer, L.D. & Reeves, W.C. 1983. Intrinsic factors affecting vector competence of mosquitoes for arbovirusses. *Annual Review of Entomology*, **28**, 229-262.

Holbrook, F.R. 1982. Evalution of three insecticides against colonized and field-collected larvae of *Culicoides varripennis* (Diptera: Ceratopogonidae). *Journal of Economic Entomology*, **75**, 736-737.

Howell, P.G. 1962. The isolation and identification of further antigenic types of African horse sickness virus. *Onderstepoort Journal of Veterinary Research*, **29**, 139-149.

Howell, P.G. 1969. The antigenic classification of strains of bluetongue virus, their significance and use in prophylactic immunization. PhD Thesis, University of Pretoria, Pretoria.

Jupp, P.G., McIntosh, B.M. & Nevill, E.M. 1980. A survey of the mosquito and *Culicoides* faunas at two localities in the Karoo region of South Africa with some observations on bionomics. *Onderstepoort Journal of Veterinary Research*, **47**, 1-6.

Kettle, D.S. 1995. Ceratopogonidae (Biting Midges), in Kettle, D.S. *Medical and Veterinary Entomology.* CAB International, Wallingford, Oxon, UK.

Linley, J.R. 1979. Nutrient agar mixtures for culture of food organisms for predaceous *Culicoides* larvae (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, **16**, 171-172.

Maclachlan, N.J. & Guthrie, A.J. 2010. Re-emergence of bluetongue, African horse sickness, and other Orbivirus diseases. *Veterinary Research*, **41**, 35.

McIntosh, B.M. 1958. Immunological types of horsesickness virus and their significance in immunization. *Onderstepoort Journal of Veterinary Research*, **27**, 465-470.

Meiswinkel, R. 1989. Afrotropical *Culicoides*: A rediscription of *C.* (Avaritia) *imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C.* (A.) *bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, **56**, 23-39.

Meiswinkel, R. 1997. Discovery of a *Culicoides imicola*-free zone in South Africa: preliminary notes and potential significance. *Onderstepoort Journal of Veterinary Research*, **64**, 81-86.

Meiswinkel, R., Baylis, M. & Labuschagne, K. 2000. Stabling and the protection of horses from *Culicoides bolitinos* (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. *Bulletin of Entomological Research*, **90**, 509–515.

Meiswinkel, R., Goffredo, M., Dijkstra, E.G.M., van der Ven, I.J.K., Baldet, T. & Elbers, A. 2008. Endophily in *Culicoides* associated with BTV-infected cattle in the province of Limburg, south-eastern Netherlands, 2006. *Preventive Veterinary Medicine*, **87**, 182–195.

Meiswinkel, R. & Linton, Y-M. 2003. Afrotropical *Culicoides* Latreille (Diptera: Ceratopogonidae): morphological and molecular description of a novel fruit-inhabiting member of the Imicola Complex, with a re-description of its sister species *C. (Avaritia) pseudopallidipennis* Clastrier. *Cimbebasia*, **19**, 37-79.

Meiswinkel, R., van Rijn, P., Leijs, P. & Goffredo, M. 2007. Potential new *Culicoides* vector of bluetongue virus in northern Europe. *The Veterinary Record*, **161**, 564-565.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, 2004, pp. 1231–1246.

Mellor, P.S., 1992. *Culicoides* as potential orbivirus vectors in Europe. In: Walton, E., Osburn, B.I. (Eds.), Bluetongue, African horse sickness, and related orbiviruses. CRC Press, Inc. Boca Raton, Florida, pp. 278-283

Mellor, P.S. 1993. African horse sickness transmission and epidemiology. *Veterinary Research*, **24**, 199-212.

Mellor, P.S. 2004. Infection of the vectors and bluetongue epidemiology in Europe. *Veterinaria Italiana*, **40**, 167-174.

Mellor, P.S. & Boorman, J. 1995. The transmission and geographical spread of African horse sickness and bluetongue viruses. *Annals of Tropical Medicine and Parasitology*, **89**, 1-15.

Mellor, P.S., Boorman, J. & Baylis, M. 2000. *Culicoides* biting midges: Their role as Arbovirus Vectors. *Annual Review of Entomology*, **45**, 307-340.

Mellor, P.S., Carpenter, S. & White, D.V. 2009. Bluetongue virus in the insect host. *Bluetongue* (ed. by P. Mellor, M. Baylis and P. Mertens) pp. 295-320. Elsevier, London.

Mellor P.S. & Hamblin C. 2004. African horse sickness. *Veterinary Research*, **35**, 445-466.

Mellor, P.S., Pitzolis, G., 1979. Observations on breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bulletin of Entomological Research*, **69**, 229–234.

Mellor, P.S., Rawlings, P., Baylis, M. & Wellby, M.P. 1998. Effect of temperature on African horse sickness virus infection in *Culicoides. Archives of Virology (Supplement)*, **14**, 155-163.

Mullens, B.A., Gerry, A.C., Lysyk, T.J. & Schmidtmann, E.T. 2004. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpretating laboratory data in a field context. *Veterinaria Italiana*, **40**, 160-166.

Mullens, B.A., Velten, R.K, Gerry, A.C., Braverman, Y. & Endris, R.G. 2000. Effects of permethrin and pirimiphos-methyl applied to cattle on feeding and survival of

Culicoides sonorensis (Diptera: Ceratopogonidae). *Medical and Veterinary Entomology*, **14**, 313-320.

Neitz, W.O. 1948. Immunological studies on bluetongue in sheep. *Onderstepoort Journal of Veterinary Science and Animal Industry*, **23**, 93-136.

Nelson, R.L. & Scrivani, R.P. 1972. Isolations of arboviruses from parous midges of the *Culicoides variipennis* complex, and parous rates in biting populations. *Journal of Medical Entomology*, **9**, 277-281.

Nevill, E.M. 1967. Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. M.Sc. (Agric) Thesis. University of Pretoria.

Nevill, E.M. 1968. A significant new breeding site of *Culicoides pallidipennis* Carter, Ingram and Macfie (Diptera: Ceratopogonidae). *Journal of the South African Veterinary and Medical Association*, **39**, 61.

Nevill, E.M. 1969. The morphology of the immature stages of some South African *Culicoides* species (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **36**, 265-284.

Nevill, E.M. 1971. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research*, **38**, 65-72.

Nevill, E.M., Erasmus, B.J. & Venter, G.J. 1992a. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae). *In* Walton, T.E. and Osburn, B.I. *Bluetongue, African horse sickness and related orbiviruses*. CRC Press, Boca Raton, Florida. 314-319.

Nevill, E.M., Venter, G.J. & Edwardes, M. 1992b. Potential *Culicoides* vectors of livestock orbivirus. In *Bluetongue, African horse sickness and related orbiviruses Proceedings of the 2nd International Symposium* (ed. by T.E.Walton and B.I. Osburn), pp. 306-313. CRC Press, Boca Raton, Florida.

Nevill, E.M., Venter, G.J., Edwardes, M., Pajor, I.T.P., Meiswinkel, R. & Van Gas, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the

Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **55**, 101-106.

Nevill, Hilda, Venter, G.J., Meiswinkel, R. & Nevill, E.M. 2007. Comparative descriptions of the pupae of five species of the *Culicoides imicola* complex (Diptera: Ceratopogonidae) from South Africa. *Onderstepoort Journal of Veterinary Research*, **74**, 97–114.

Nevill, Hilda, Nevill, E.M. & Venter, G.J. 2009. Description and comparison of the pupae of a further two *Culicoides* (*Avaritia*) species from the dung of large herbivores in South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **76**, 277–284.

Nunamaker, R.A., Sieburth, P.J., Dean, V.C., Wigington, J.G., Nunamaker, C.E. & Mecham, J.O. 1990. Absence of transovarial transmission of bluetongue virus in *Culicoides variipennis*: immunogold labeling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). *Comparative Biochemistry and Physiology*, **96**, 19-31.

Page, P.C., Labuschagne, K., Nurton, J.P., Venter, G.J. & Guthrie, A. 2009. Duration of repellency of N,N-diethyl-3-methylbenzamide, citronella oil and cypermethrin against *Culicoides* species when applied to polyester mesh. *Veterinary Parasitology*, **163**, 105–109.

Paweska, J.T., Prinsloo, S. & Venter, G.J. 2003. Oral susceptibility of South African *Culicoides* species to live-attenuated serotype-specific vaccine strains of African horse sickness virus (AHSV). *Medical and Veterinary Entomology*, **15**, 436-447.

Paweska, J.T., Venter, G.J. & Mellor, P.S. 2002. Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV 1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Medical and Veterinary Entomology*, **16**, 10-21.

Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C. & Baylis, M. 2005. Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology*, **3**, 171–181.

Saegerman, C., Berkvens, D. & Mellor, P.S. 2008. Bluetongue epidemiology in the European Union. *Emerging Infectious Diseases,* **14**, 538–544.

Schwartz-Cornil, I., Mertens, P.P., Contreras, V., Hemati, B., Pascale, F., Bréard, E., Mellor, P.S., MacLachlan, N.J. & Zientara, S. 2008. Bluetongue virus: virology, pathogenesis and immunity. *Veterinary Research,* **39**, 46.

Sebastiani, F., Meiswinkel, R., Gomulski L.M., Guglielmino, C.R., Mellor, P.S., Malacrida, A.R., Gasperi, G. 2001. Molecular differentiation of the Old World *Culicoides imicola* species complex (Diptera, Ceratopogonidae) inferred using random amplified polymorphic. DNA markers. *Molecular Ecology,* **10,** 1773-1786.

Sellers, R.F. 1992. Weather, *Culicoides*, and the distribution and spread of bluetongue and African horse sickness viruses. *In* Walton, T.E. and Osburn, B.I. *Bluetongue, African Horse Sickness and Related Orbiviruses*. CRC, Boca Raton, Florida.

Sellers, R.F., Pedgley, D.E. & Tucker, M.R. 1977. Possible spread of African horse sickness on the wind. *Journal of Hygiene, Cambridge,* **79**, 279-298.

Standfast, H.A & Dyce, A.L. 1972. Potential vectors of arboviruses of cattle and buffalo in Australia. *Australian Veterinary Journal*, **48**, 224-227.

Tabachnick, W.J. 1991. Genetic control of oral susceptibility of *Culicoides variipennis* with bluetongue virus. *American Journal of Tropical Medicine and Hygiene,* **45**, 666-671.

Tabachnick, W.J. 2004. *Culicoides* and the global epidemiology of bluetongue virus infection. *Veterinaria Italiana,* **40**, 145-150.

Takamatshu, H., Mellor, P.S., Mertens, P.P.C., Kirkham, P.A., Burroughs, J.N., Parkhouse, R.M.E. 2003. A possible overwintering mechanism for bluetongue virus in the absence of the insect vector. *Journal of General Virology*, **84**, 227-235.

Thiry, E., Saegerman, C., Guyot, H., Kirten, P., Losson, B., Rollin, F., Bodmer, M., Czaplicki, G., Toussaint, J.F., de Clercq, K., Dochy, J-M, Dufey, J., Gilleman, J-L. & Messeman, K. 2006. Bluetongue in northern Europe. *The Veterinary Record*, **159**, 327.

Trigg, J.K. 1996. Evaluation of a eucalyptus-based repellent against *Culicoides impunctatus* (Diptera: Ceratopogonidae) in Scotland. *Journal of the American Mosquito Control Association,* **12**, 32–330.

Van Dijk, A.A. 1998. African horsesickness vaccine development. In *Proceedings of the Eight International Conference on Equine Infectious Diseases* (ed. by U. Wernery, J.F. Wade, J.A. Mumford and O.-R. Kaaden), pp. 261-379. 23-26 March 1998, United Arab Emirates, Dubai.

Venter, G.J., Gerdes, G.H., Mellor, P.S. & Paweska, J.T. 2004. Transmission potential of South African *Culicoides* species for live-attenuated bluetongue virus. *Veterinaria Italiana,* **40**, 198-203.

Venter, G.J., Hamblin, C. & Paweska, J.T. 2003. Determination of the oral susceptibility of South African livestock-associated *Culicoides* species, to bovine ephemeral fever virus. *Medical and Veterinary Entomology*, **17**, 133-137.

Venter, G.J., Koekemoer, J.J.O. & Paweska, J.T. 2006. Investigations on outbreaks of African horse sickness in the surveillance zone of South Africa. *Revue scientifique et technique, Office International des Epizooties,* **25**, 1 097-1109.

Venter, G.J., Labuschagne, K., Boikanyo, S.N.B., Morey, L. & Snyman, M.G. 2011. The repellent effect of organic fatty acids on *Culicoides* midges as determined with suction light traps in South Africa. *Veterinary Parasitology,* **181**, 365-369.

Venter, G.J. & Meiswinkel, R. 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort Journal of Veterinary Research*, **61**, 327-340.

Venter, G.J., Nevill, E.M. & Van der Linde, T.C. 1996. Geographical distribution and relative abundance of stock associated *Culicoides* species (Diptera: Ceratopogonidae) in southern Africa, in relation to their potential as viral vectors. *Onderstepoort Journal of Veterinary Research*, **63**, 25-38.

Venter, G.J., Nevill, E.M. & Van der Linde, T.C. 1997. Seasonal abundance and parity of stock-associated *Culicodies* species (Diptera: Ceratopogonidae) in different

climatic regions in southern Africa in relation to their viral vector potential. *Onderstepoort Journal of Veterinary Research*, **64**, 259-271.

Venter, G.J. & Paweska, J.T. 2007. Virus recovery rates for wild-type and live attenuated vaccine strains of African horse sickness virus serotype 7 in orally infected South African *Culicoides* species. *Medical and Veterinary Entomology*, **21**, 377-383.

Venter, G.J., Paweska, J.T. van Dijk, A.A., Mellor, P.S. & Tabachnick, W.J. 1998. Vector competence of *Culicoides bolitinos* and *C. imicola* (Diptera: Ceratopogonidae) for South African bluetongue virus serotypes 1, 3 and 4. *Medical and Veterinary Entomology*, **12**, 101-108.

Venter, G.J., Paweska, J.T., Williams, R. & Nevill, E.M. 1999. Prevalence of antibodies against African horse sickness (AHS) and equine encephalosis (EE) viruses in donkeys in southern Africa. In: Werney, U., Wade, J.F., Mumford, J.A. & Kaaden, O.R. (eds). 1999. *Proceedings Eighth International Conference on Equine infectious diseases*. R & W Publication (Newmarket) Limited, Newmarket. pp 299-302

Venter, G.J., Wright, I.M., van der Linde, T.C. & Paweska, J.T. 2009. The oral susceptibility of South African field populations of *Culicoides* to African horse sickness virus. *Medical and Veterinary Entomology*, **23**, 367–378.

Veronesi, E., Venter, G.J., Labuschagne, K., Mellor, P.S. & Carpenter, S. 2009. Life-history parameters of *Culicoides* (*Avaritia*) *imicola* Kieffer in the laboratory at different rearing temperatures. *Veterinary Parasitology*, **163**, 370-373

Verwoerd, D.W. & Erasmus, B.J. 2004. Bluetongue, in: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, pp. 1201–1220.

Walker, A.R. & Davies, F.G. 1971. A preliminary survey of the epidemiology of bluetongue in Kenya. *Journal of Hygiene, Cambridge*, **69**, 47-60.

Walton, T.E. 2004. The history of bluetongue and a current global interview. *Veterinaria Italiana*, **40**, 31-38.

Wellby, M.P., Baylis, M., Rawlings, P. & Mellor, P. 1996. Effect of temperature on the rate of virogenesis of African horse sickness virus in *Culicoides* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. *Bulletin of Entomological Research*, **86**, 715-720.

Wittmann, E.J., Mellor, P.S. & Baylis, M. 2001. Using climate data to map the potential distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Revue scientifique et technique, Office International des Epizooties*, **20**, 731-740.

2. EVALUATION OF THE NEWLY DEVELOPED FREE STATE SUCTION LIGHT TRAP FOR COLLECION OF *CULICOIDES* MIDGES

2.1 Introduction

The presence of competent *Culicoides* vectors is critical for the spread, occurrence and maintenance of AHSV and BTV in an area. The detection of all potential vectors of these viruses will be crucial to the implementation of integrated control measures, disease risk analysis and the effective management of these diseases (*e.g*. the determination of vector-free periods and/or areas). The collection and identification of *Culicoides* midges is an important prerequisite in understanding, monitoring and predicting disease spread. In most surveillance systems or studies involving *Culicoides*, the principal aim will be to capture the maximum number of potential vectors near their vertebrate hosts.

Various models of light traps have been used since 1928 for the collection and monitoring of night-active insects (Service, 1977). Despite the relative importance of the collection and monitoring of midges only a limited number of suction light traps, the primary monitoring tool used for this purpose, are commercially available. The majority of these traps, *e.g*. the CDC light trap, were originally designed for the collection of mosquitoes. The light trap has become the recommended tool for the collection of *Culicoides*, even though this is an artificial collection system and despite the great variety of factors that can influence light trap results (Mellor *et al.*, 2004).

Light traps are routinely used to determine the risk of a virus moving into, becoming established and spreading in an area, and also to determine times and areas of low risk for disease transmission (Goffredo *et al*., 2004; Patakakis, 2004; Cagienard *et al*., 2006; Meiswinkel *et al*., 2008; Racloz *et al*., 2008). In the absence of laboratory colonies of some vectors, light traps are used for the collection of live *Culicoides* to be used in further studies of vector capacity and disease transmission (Paweska *et al.*, 2003; 2005; Veronesi *et al*., 2009; Venter *et al*., 2011).

Suction light traps are very effective and not labour intensive as they are put up from dusk till dawn and will attract midges in the area. To date the most efficient suction

light trap used, and even the OIE benchmark for light traps is the Onderstepoort 220V down draught black light trap (Mellor *et al*., 2004). This is supported by the results of a comparative study done in South Africa which showed that the Onderstepoort black-light trap collects significantly more *Culicoides* midges than the Rieb, mini-CDC, Pirbright and BG-sentinel light traps, under field conditions (Venter *et al*., 2009). Taking into account the more powerful light source and fan of the Onderstepoort trap, compared to that of the others, this result was not surprising.

The development and evaluation of more efficient light traps will form an important role in monitoring vector populations. In the present study the efficiency of a newly developed black light Free State trap (FS Trap) (Fig. 2.1a) for the collection of *Culicoides* midges was compared to that of the Onderstepoort trap (OP Trap) (Fig. 2.1b). As *Culicoides* collections are sometimes done in remote areas where 220V electricity is not available the efficacy of 220V and 12V traps, of both models were evaluated.

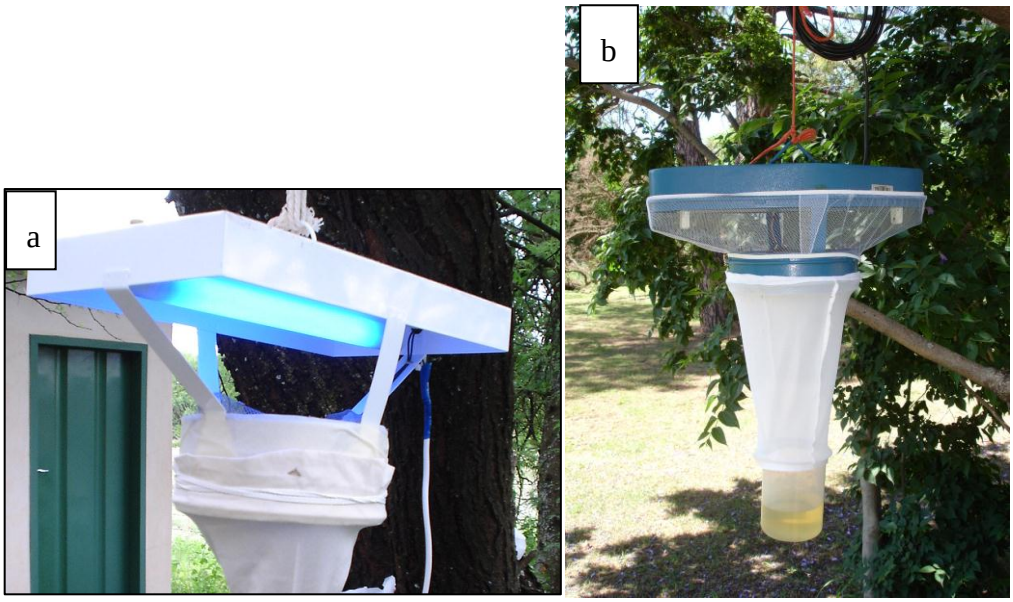

Figure 2.1 The Free State 220V (FS trap) (a) and Onderstepoort 220V (OP trap) (b) down draught suction light traps.

2.2 Material & Methods

2.2.1 Light traps

The FS and OP traps are similar in design. Both traps make use of a light source to attract insects to a fan that sucks them into a collecting beaker underneath the light source and fan. In the FS trap the distance between the light source and the fan is greater than that of the OP trap. In the FS trap the netting to keep moths and other larger insects out of the collections is installed directly over the fan as opposed to around the entrance of the trap as in the OP one. All the traps were fitted with 8W UV light tubes. In the two OP traps black light tubes, which filter out most visible light, were used. Both light sources, however, emitted UV light in the range of 300-400 nm. The 12V traps were powered by 12 V, 105 Ah sealed lead-acid battery.

2.2.2 Trap comparisons

The traps were compared on 12 nights from 27 February to 14 March 2008 at four sites at the ARC-Onderstepoort Veterinary Institute (25°39'S, 28°11'E; 1219 m above sea level).

Traps were deployed in three replicates of a 4 x 4 randomized Latin square design (Snedecor & Cochran, 1980). The advantage being that each treatment occurs once at each site and on each occasion the treatment means would be independent of any effects due to site or occasion and, as only one treatment occupies a site on any occasion, trap interaction was also avoided (Perry *et al*., 1980).

Traps were operated from dusk to dawn at opposite ends of open-sided barns housing between 20 and 40 cattle each. Traps were hung 1.4m above ground level and as close to the cattle as practically possible. To prevent interference between traps all were located at least 15 m apart. Insects were collected into water to which 0.5% 'Savlon' (Johnson & Johnson, South Africa) (contains Clorhexidine gluconate 0.3 g/100 ml and Cetrimide 3.0 g/100 ml) antiseptic had been added to break the surface tension of the water and allow midges to sink to the bottom. After retrieval in the morning, the insects were transferred to 70% ethanol and stored in the dark at 4°C until analysed (Goffredo & Meiswinkel, 2004).

2.2.3 Sub sampling

Where collections consisted of more than 500 midges, sub samples were made according to the method of Van Ark & Meiswinkel (1992). This method consists of five steps: 1. The *Culicoides* are separated from non-*Culicoides*. 2. The *Culicoides* are then suspended in a known volume of 70% ethanol. 3. A sub sample of a known volume is then taken from the middle of the suspension with a pipette with an aperture of at least 2 mm. 4. All midges in the sub sample are counted and identified. 4. Sub sampling is continued until at least 500 specimens are identified. 5. The volume of the sub sample in relation to the volume of the total collection is determined and used for the calculation of the estimated total catch.

All *Culicoides* midges in the sub sample were identified to species level using the wing picture atlas of Afro-tropical *Culicoides* (R. Meiswinkel 1994, unpublished data). The midges were also sexed and the females were graded in four different gonotrophic age structure categories according to the abdominal pigmentation method of Dyce (1969). The categories were: nulliparous or unpigmented (Fig.2.2 a), parous or pigmented (Fig.2.2 b), freshly blood-fed (Fig 2.2 c) and gravid females with eggs visible in the abdomen.

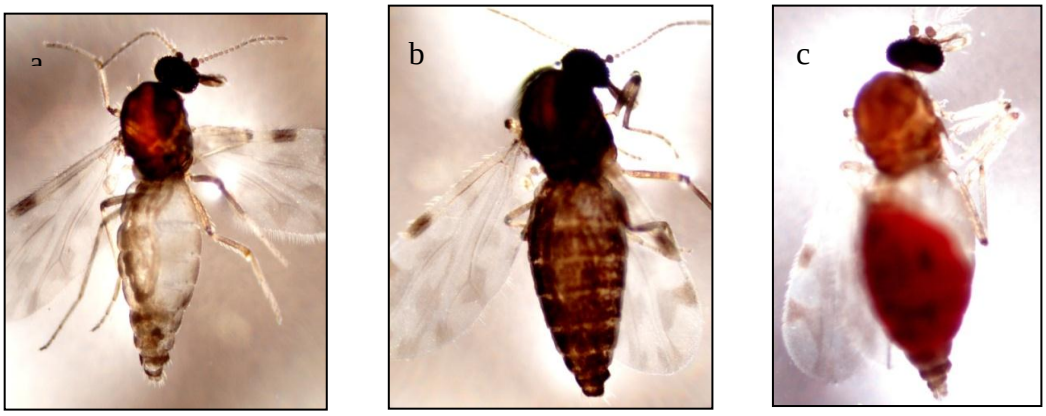

Figure 2.2 (a) Nulliparous female, (b) parous female and (c) blood-fed female *C. imicola*

2.2.3 Statistical analyses

Analysis of variance (ANOVA) was used to differentiate between the trap treatment effects. The data were normally distributed and had heterogeneous treatment variances and testing was done at the 5% level. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level of

significance (Snedecor & Cochran, 1980). Data were analysed using the statistical program GenStat10 (Payne *et al*., 2007). Species diversity at each trapping site was calculated with the Shannon Weiner index, which describes the evenness in distribution of species abundances taking sample size into account.

2.3 Results

The average number and the variation in these numbers collected by each of the traps can be seen in Table 2.3.1. A total of 2 131 694 *Culicoides* midges belonging to nine species were collected in 48 collections made with the four traps over 12 nights from 27 February to 14 March 2008 (Table 2.3.1). Of the total number collected 51.3% (1 095 555) were collected with the OP 220V trap while the 12V collected 28.1% (599 242). The FS 12V and 220V collected respectively 10.7% (229 082) and 9.8% (210 086) of the total number of midges.

Taking into account the day to day variation in the collection size the average number of midges (91 296.3) collected with the OP 220V trap (Table 2.3.1) was significantly higher than that of the OP 12V trap ($P = 0.029$), the FS 220V ($P < 0.001$) or the FS 12V trap ($P < 0.001$). The average numbers of midges collected with the OP 12V trap did not differ significantly from that of the FS 220V ($P = 0.057$) but was significantly higher than collected in the FS 12V ($P = 0.019$). The average number of midges collected with the FS 220V trap did not differ significantly from that of the FS 12V trap ($P = 0.189$).

A total of nine different *Culicoides* species were collected in this survey (Table 2.3.2). The species richness varied from seven as determined with the FS 220V and OP 12V to only four as determined with the FS 12V (Table 2.3.2). The species diversity ranged from H = 0.09 (OP 220V) to H = 0.16 (FS 220V) (Table 2.3.2). Variations in species richness and species diversity between treatments were the result of single specimens of some species which were collected on only a few trapping occasions (Table 2.3.2). For example *Culicoides nivosus* and *C. gulbenkiani* were collected on only one occasion in respectively the OP 220V and OP 12V traps. Both these species form less than 0.1% of the total number of midges collected (Table 2.3.2).

The dominant *Culicoides* species collected in all four traps was *C. imicola.* As with the average number the OP 220V trap collected significantly more *C. imicola* than the

OP 12V trap ($P = 0.030$), the FS 220V ($P < 0.001$) or the FS 12V trap ($P < 0.001$). The average number of *C. imicola* collected by the OP 12V trap was not significantly higher than collected with the FS 220V ($P = 0.050$) but did differ significantly from that of the FS 12V ($P = 0.019$). Once again the number of *C. imicola* collected with the FS 220V trap did not differ significantly from that collected with the FS 12V trap ($P = 0.189$).

In all four traps *C. enderleini* was found to be the second most dominant species collected. Similar to *C. imicola*, the average number of *C. enderleini* collected with the OP 220V trap was significantly higher than the OP 12V trap ($P = 0.030$) the FS 220V ($P = 0.001$) and the FS 12V trap ($P = 0.002$). Similarly the OP 12V trap did not collect significantly more *C. enderleini* than the FS 220V trap ($P = 0.057$) but did collect more than the FS 12V trap ($P = 0.039$). The numbers of *C. enderleini* collected with the two FS traps did not differ significantly ($P = 0.378$).

The proportion of the dominant species, *C. imicola*, in the different trap collections ranged from 96.7% (OP 12V trap) to 98.4% (OP 220V trap) (Table 2.3.1.). The OP 12V trap collected a significantly smaller proportion of *C. imicola* than the OP 220V trap ($P = 0.035$), none of the other traps differed significantly.

As expected for light traps females were the dominant gender collected. The proportion of *C. imicola* females ranged from 93.7% (FS 12V) to 97.7% (OP 220V). In *C. enderleini* this percentage ranged from 82.9% (FS 220V) to 90.3% (FS 12V). The proportion of both *C. imicola* and *C. enderleini* females collected in relation to the total number of males collected with all four traps were significantly different ($P < 0.001$ for both species).

The proportion nulliparous *C. imicola* collected by the OP 12V trap was significantly higher than that of the OP 220V trap ($P < 0.001$), FS 220V trap ($P < 0.001$) and the FS 12V trap ($P < 0.001$). In fact the proportion of nulliparous *C. imicola* differed significantly between all the traps evaluated.

The nulliparous *C. enderleini* as determined by the different traps ranged from 29.4% (FS 220V) to 45.9% (FS 12V) (Table 2.3.1). The proportion of nulliparous *C. enderleini* females collected with the four traps differed significantly from each other

with the exception of the OP 220V and FS 220V traps that did not differ significantly ($P = 0.474$).

The parous *C. imicola* collected by the different traps ranged from 42.9% (OP 12V) to 48.3% (FS 12V) (Table 2.3.1). The proportion of parous *C. imicola* collected with the four traps differed significantly from each other with the exception of the OP 220V and FS 12V traps that did not differ significantly ($P = 0.708$).

The parous *C. enderleini* collected by the different traps ranged from 38.4% (FS 12V) to 57.6% (OP 220V) (Table 2.3.1). The proportion of parous *C. enderleini* collected with the four traps differed significantly from each other with the exception of the OP 12V and FS 220V traps that did not differ significantly ($P = 0.222$).

The freshly blood-fed rate of *C. imicola* collected by the different traps ranged from 0.1% (FS 12V) to 4.1% (OP 220V) (Table 2.3.1). The proportion of freshly blood-fed *C. imicola* females differed significantly between all four traps evaluated.

The gravid *C. imicola* in the different traps ranged from 1.7% (FS 12V) to 2.8% (FS 220V) (Table 2.3.1). The proportion of gravid *C. imicola* collected by the four traps differed significantly from each other with the exception of the OP 220V and FS 12V traps that did not differ significantly ($P = 0.275$).

The gravid *C. enderleini* in the different traps ranged from 0% (OP 220V) to 6.01% (FS 12V) (Table 2.3.1). The proportion of gravid *C. enderleini* in the four traps differed significantly from each other with the exception of the two FS traps that did not differ significantly ($P = 0.248$).

Table 2.3.1 Gonotrophic age stucture and sex ratio of *Culicoides* species collected using the FS 12V, FS 22V, OP 12V and OP 220V suction light traps at the ARC-OVI on 12 nights from 27 February to 14 March 2008

Trap	**OP 220V**		**OP 12V**		**FS 220V**		**FS 12V**	
No. of collections made	12		12		12		12	
No. of species collected	5		7		7		4	
Total *Culicoides* collected (%)	1 095 555	(51.3)	599 242	(28.1)	210 086	(9.8)	229 082	(10.7)
Average collection size	91 296.3 a		49 936.8 b		17 507.2 b, c		19 090.2 c	
Range in collection size	13 050-180 450		1 390-165 600		1 990-67 200		2 540-122 040	
Comparison with OP 220V trap	100%		54.7%		19.2%		20.9%	
Age grading results								
C. imicola								
Mean Collected (%)	89 785.4 a	(98.4)	48 296.1 b	(96.7)	16 949.4 b, c	(96.8)	18 556.4 c	(97.2)
Mean Nulliparous (%)	39 145.3 a	(43.6)	24 797.3 b	(51.3)	8 145.8 c	(48.1)	7 919.4 d	(42.7)
Mean Parous (%)	43 254.4 a	(48.2)	20 718.8 b	(42.9)	7 632.9 c	(45.0)	8 968.4 a, d	(48.3)
Mean Freshly blood fed (%)	3 679.5 a	(4.1)	61.7 b	(0.1)	108.3 c	(0.6)	177.1 d	(1.0)
Mean Gravid (%)	1 666.7 a	(1.9)	975.5 b	(2.0)	468.7 c	(2.8)	322.3 a, d	(1.7)
Mean Males (%)	2 039.5 a	(2.3)	1 742.9 b	(3.6)	593.7 b, c	(3.5)	1 169.3 d	(6.3)
C. enderleini								
Mean Collected (%)	1 256.8 a	(1.4)	1 591.7 b	(3.2)	445.7 b, c	(2.6)	527.1 c	(2.8)
Mean Nulliparous (%)	394.0 a	(31.4)	595.5 b	(37.4)	131.1 a, c	(29.4)	242.1 d	(45.9)
Mean Parous (%)	724.2 a	(57.6)	730.9 b	(45.9)	219.6 b, c	(49.3)	202.2 d	(38.4)
Mean Freshly blood fed (%)	0.0		0.0		0.0		0.0	
Mean Gravid (%)	0.0		35.3 a	(2.2)	18.8 b	(4.2)	31.7 b, c	(6.0)
Mean Males (%)	138.7 a	(11.0)	229.9 b	(14.5)	76.3 b, c	(17.1)	51.2 a, d	(9.7)

Significant differences between traps are indicated by a letter (a, b, c or d) next to the average numbers.

Table 2.3.2 A summary of the *Culicoides* species collected using the FS 12V, FS 220V, OP 12V and OP 220V suction light traps at the ARC-OVI on 12 nights from 27 February to 14 March 2008

Trap	**OP 220V**		**OP 12V**		**FS 220V**		**FS 12V**		Total collected in all four traps
	Frequency in 12 collections	Total Collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 Collections	Total collected (%)	
C. imicola	12	1 077 425 (98.3)	12	579 553 (96.7)	12	203 393 (96.8)	12	222 677 (97.2)	2 083 048 (97.6)
C. enderleini	10	15 082 (1.4)	12	19 100 (3.2)	12	5 348 (2.5)	12	6 325 (2.8)	45 855 (2.1)
C. bolitinos	4	2178 (0.2)	4	129 (<0.1)	3	900 (0.4)	2	60 (<0.1)	3267 (0.2)
C. milnei	2	460 (<0.1)	1	20 (<0.1)	4	275 (0.1)	1	20 (<0.1)	775 (<0.1)
C. nivosus	1	410 (<0.1)							410 (<0.1)
C. magnus			1	320 0.1	1	80 (<0.1)			400 (<0.1)
C. zuluensis			1	100 (<0.1)	1	40 (<0.1)			140 (<0.1)
C. leucostictus					2	50 (<0.1)			50 (<0.1)
C. gulbenkiani			1	20 (<0.1)					20 (<0.1)
Species diversity (H)	0.09		0.15		0.16		0.13		
Total	1 095 555		599 242		210 086		229 082		2 133 965

The *C. imicola* males as determined by the different traps ranged from 2.3% (OP 220V) to 6.3% (FS 12V) (Table 2.3.1). The proportion of *C. imicola* males in the four traps differed significantly from each other with the exception of the OP 12V and FS 220V traps that did not differ significantly ($P = 0.544$).

The *C. enderleini* males in the different traps ranged from 9.7% (FS 12V) to 17.1% (FS 220V) (Table 2.3.1). The proportion of *C. enderleini* males differed significantly from each other with the exception of the OP 220V and FS 12V ($P = 0.402$) and the OP 12V and FS 220V ($P = 0.194$) traps that did not differ significantly.

2.4. Discussion

All traps evaluated collected *Culicoides* midges on every night they were operated. All four traps furthermore found *C. imicola* to be the most abundant and *C. enderleini* to the second most abundant species. The OP 220V trap collected significantly more midges than any of the others. In fact, more than half of the midges collected in this survey were collected with the OP 220V trap. Since all four traps have the same basic design these differences were unexpected. The main difference between the FS and OP traps is the distance between the light source and the fan and this seems to be crucial when the results are taken into account. The OP trap was originally designed as a 220V trap and the current result shows clearly that the change to 12V did negatively influence the effectiveness. These results indicate that relatively small changes in trap design can have significant influences on the effectiveness.

The FS 220V and OP 12V trap did, however, collect more species than the others. When collecting and monitoring *Culicoides* midges for evaluating risk of disease transmission, species diversity is an important factor to consider as BTV and AHSV can potentially be transmitted by multiple vector species (Paweska *et al.*, 2003; Mellor *et al.*, 2009; Venter *et al.*, 2011). In the present study sub samples of 500 midges were analyzed and in some cases, this represented < 0.3% of the midges collected on a single occasion and increased the probability that less abundant species could have been missed. Not making sub samples would, however, have rendered it nearly impossible to identify and categorize the more than two million midges collected in a reasonable time frame. In the absence of laboratory colonies, however,

higher numbers of live midges will be collected by the OP 220V trap for studies involving the use of live midges.

Since it is accepted that transovarial transmission of virusses does not occur in the genus *Culicoides* (Nelson & Scrivani, 1972; Nunamaker *et al.,* 1990) the gonotrophic age structure of a *Culicoides* population can be an important indicator of the population's potential vector status and the number of parous midges will be of importance. In this context it is worth mentioning that Braverman & Mumcuoglu (2009) indicated that pigmentation may not be linked to the ingestion of a bloodmeal and oogenesis as previously accepted. In the present study the parous rate in *C. imicola* as determined by the various traps varied between 42.9% and 48.3%. Although these differences were found to be statistically significant, all four traps gave comparable results.

These results highlight the extreme caution that needs to be exercised in the comparison and interpretation of light trap data. It must be remembered that it reflects only a small portion of the midges actively seeking blood in the vicinity and that this portion is deemed to be < 0.0001% (Meiswinkel *et al.,* 2004) and is influenced by a variety of factors. A major drawback of light traps is the low number of males, gravid and blood-fed females collected. Service (1971) suggested that this might be a result of physiology or the attraction of these groups to livestock or even factors such as differences in mortality between the sexes. Light traps will also not sample diurnal *Culicoides* species that may play an important role in the epidemiology of BT but have not been regarded as relevant (Nathan, 1981).

The number of midges collected in a light trap in an area is not an indication of the biting rate (Carpenter *et al.,* 2008; Gerry *et al*., 2009; Viennet *et al.,* 2011) but is only an indication of the numbers and species present in the area. Despite the great number of factors affecting light trap collections, they are still the most effective and reliable method to collect and monitor the relative abundance and seasonality of *Culicoides* midges.

Although the FS 12V and 220V traps were less effective in collecting a large number of *Culicoides* midges, they did collect relatively large numbers and in the same proportion as the OP 220V trap. The FS traps collected about 20% of the midges

collected by the OP 220V trap and this can be regarded as a sub sample of the OP 220V traps collection. Thus when not identifying the entire sample of midges, there is little use in collecting the enormous numbers of *Culicoides* midges which are collected using the OP 220V trap.

In contrast to the OP 220V and 12V traps it was shown that there was no significant difference in the numbers of midges collected with the two FS traps. The results of these two were comparable and these traps can easily be interchanged between sites. Due to the fact that the FS 12V trap can be used in the absence of a 220V power supply it can be used over a much wider area. It was therefore decided to use the FS traps and to compare the results obtained in further experiments to determine some of the factors influencing the numbers of midges in light traps and even some behavioral patterns.

2.5. References

Braverman, Y. & Mumcuoglu, K. 2009. Newly emerged nulliparous *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae) with pigmented abdomen. *Veterinary Parasitology*, **160**, 356-358.

Cagienard, A., Griot, C., Mellor, P.S., Denison, E. & Stark, K.D.C. 2006. Bluetongue vector species of *Culicoides* in Switzerland. *Medical and Veterinary Entomology*, **20**, 239–247.

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. & Mellor, P. 2008. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector? *Journal of Applied Ecology*, **45**, 1237-1245.

Dyce, A.L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society*, **8**, 11-15.

Gerry, A.C., Monteys, V.S.I., Vidal, J.M., Francino, O. & Mullens, B.A. 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in Northeastern Spain in relation to midge capture using UV light and carbon dioxide-baited traps. *Journalof Medical Entomology*, **46**, 615-624.

Goffredo, M. & Meiswinkel, R. 2004. Entomological surveillance of bluetongue in Italy: methods of capture, catch analysis and identification of *Culicoides* biting midges. *Veterinaria Italiana,* **40**, 260-265.

Goffredo, M., Conte, A. & Meiswinkel, R. 2004. Distribution and abundance of *Culicoides imicola, obsoletus* complex and *pulicaris* complex (Diptera: Ceatopogonidae) in Italy. *Veterinaria Italiana,* **40**, 270–273.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, 2004, pp. 1231–1246.

Meiswinkel, R., Goffredo, M., Leijs, P. & Conte, A. 2008. The *Culicoides* 'snapshot': A novel approach used to assess vector densities widely and rapidly during the 2006 outbreak of bluetongue (BT) in The Netherlands. *Preventive Veterinary Medicine,* **87**, 98-118.

Mellor, P.S., Tabachnick, W., Baldet, T., Baylis, M., Bellis, G., Calistri, P., Delécolle, J-C., Goffredo, M., Meiswinkel, R., Mullens, B., Scaramozzino, P., Torina, A., Venter, G. & White, D. 2004. Conclusions of working groups. Group 2. Vectors. Proceedings of the 3rd OIE Bluetongue International Symposium, 26–29 October 2003, Taormina, Italy. *Veterinaria Italiana*, **40**, 715–717.

Mellor, P.S., Carpenter, S. & White, D.M. 2009. Bluetongue virus in the insect vector. In: Mellor, P.S., Baylis, M. &Mertens, P.C. (eds) *Bluetongue.* Elsevier, Paris: 295-320.

Nathan, M.B. 1981. A study of the diurnal biting and flight activity of *Culicoides phlebotomus* (Williston) (Diptera: Ceratopogonidae) using three trapping methods. *Bulletin of Entomological Research*, **71**, 121-128.

Nelson, R.L. & Scrivani, R.P. 1972. Isolations of arboviruses from parous midges of the *Culicoides variipennis* complex, and parous rates in biting populations. *Journal of Medical Entomology*, **9**, 277-281.

Nunamaker, R.A., Sieburth, P.J., Dean, V.C., Wigington, J.G., Nunamaker, C.E. & Mecham, J.O. 1990. Absence of transovarial transmission of bluetongue virus in

Culicoides variipennis: immunogold labeling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). *Comparative Biochemistry and Physiology*, **96**, 19-31.

Patakakis, M.J. 2004. *Culicoides imicola* in Greece. *Veterinaria Italiana*, **40**, 232–234.

Paweska, J.T., Prinsloo, S. & Venter, G.J. 2003. Oral susceptibility of South African *Culicoides* species to live-attenuated serotype-specific vaccine strains of African horse sickness virus (AHSV). *Medical and Veterinary Entomology*, **15**, 436-447.

Paweska, J.T., Venter, G.J. & Hamblin, C. 2005. A comparison of the susceptibility of *Culicoides imicola* and *C. bolitinos* to oral infection with eight serotypes of epizootic haemorrhagic disease virus. *Medical and Veterinary Entomology*, **19**, 200-207.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M., 2007. GenStat for Windows® (10th ed.). Introduction. VSN International, Hemel Hempstead, U.K.

Perry, J.N., Wall, C. & Greenway, A.R. 1980. Latin square designs in field experiments involving sex attractants. *Ecological Entomology*, **5**, 385–396.

Racloz, V., Venter, G.J., Griot, C. & Stark, D.C. 2008. Estimating the temporal and spatial risk of bluetongue related to the incursion of infected vectors into Switzerland. *BMC Veterinary Research*, **4**, 42-52.

Service, M.W., 1971. Adult flight activities of some British *Culicoides* species. *Journal of Medical Entomology*, **8**, 605-609.

Service, M.W. 1977. A critical review of procedures for sampling populations of adult mosquitoes. *Bulletin of Entomological Research*, 67, 343-382.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical Methods, 7th edition. Iowa State University Press.

Van Ark, H. & Meiswinkel, R. 1992. Sub sampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **59**, 183-189.

Venter, G.J., Labuschagne, K., Hermanides, K.G., Boikanyo, S.N.B., Majatladi, D.M. & Morey L. 2009. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Veterinary Parasitology*, **166**, 299-307.

Venter, G.J., Wright, I., Del Rio, R., Lucientes, J. & Miranda, M.A., 2011. The susceptibility of *Culicoides imicola* and other South African livestock-associated *Culicoides* species to infection with bluetongue virus serotype 8. *Medical and Veterinary Entomology*, **25**, 320-326.

Veronesi, E., Venter, G.J., Labuschagne, K., Mellor, P.S. & Carpenter, S. 2009. Life-history parameters of *Culicoides* (*Avaritia*) *imicola* Kieffer in the laboratory at different rearing temperatures. *Veterinary Parasitology*, **163**, 370-373.

Viennet E., Garros C., Lancelot R., Allene X., Gardes L., Rakotoarivony I., Crochet D., Delecolle J.C., Moulia C., Baldet T. & Balenghien T. 2011. Assessment of vector/host contact: comparison of animal-baited traps and UV-light/suction trap for collecting *Culicoides* biting midges (Diptera: Ceratopogonidae), vectors of Orbiviruses. *Parasites and Vectors*, **4**, 119-131.

3. FACTORS INFLUENCING LIGHT TRAP EFFICIENCY FOR THE COLLECTION OF *CULICOIDES* SPECIES

3.1 Introduction

One of the first steps in identifying factors influencing the occurrence and spread of pathogens transmitted by *Culicoides* midges involves the capture of maximum numbers of all potential vectors near host animals. The international status of *Culicoides*-borne diseases, however, calls for the standardization of methods to monitor midge populations in order to obtain comparable data for risk predictions (Mellor *et al.,* 2004). Standardization of methods to determine vector presence and abundance will be an essential prerequisite to determine the relative risk of outbreaks occurring in an area and in the implementation of effective control measures. To date only a limited number of trapping tools, mostly suction light traps, are used for this purpose. It was shown in Chapter 2 that although most of these traps utilized the same basic principles, huge differences in trapping efficiency could occur between different traps (Venter *et al.,* 2009b). It will therefore be important to optimize this monitoring tool for optimal efficacy.

Suction light traps have proven to be a very effective and practical method to sample midge populations in an area (Venter *et al.,* 1996; 2006). Light traps intercept only a relatively small portion of the active blood-seeking female midges in their immediate vicinity. The exact size of this portion is not known but is thought to be < 0.0001% (Meiswinkel *et al*., 2004). The number of midges collected with a light trap will not only depend on the trap, weather conditions and other variables but also on proper trap location in relation to the host together with other environmental factors (Service, 1977). Several studies have emphasized the different results for *Culicoides* abundance determined by light traps compared to various non-attractant collection methods (Carpenter *et al.,* 2008; Gerry *et al.,* 2009). Greater awareness of the factors that may influence light trap efficiency, and the incorporation of these factors into the design of light traps, can contribute to the improvement and wider implementation of data obtained.

It is well known that black or UV light will attract more night-flying insects, including *Culicoides* midges, than white light (Rowley & Jorgensen, 1967; Venter & Hermanides, 2006). The use of light traps with incandescent globes is the preferred tool used to monitor the presence and abundance of *Culicoides* species for research in Australia (Bishop *et al*., 2004a). Improved light trapping of mosquitoes has been achieved by determining mosquito responses to the colour and intensity of light (Bishop *et al*., 2004a). Insects generally perceive and respond to light in the 350-700 nm range and their relative responses can vary considerably over this range (Bishop *et al*., 2004a). Incandescent light sources used in traps generally have a maximum output at 700 nm with little or no output below 400 nm (Bishop *et al*., 2004a). Advances in light emitting diodes (LEDs) have produced energy efficient LEDs that may become more effective than incandescent lights and more suitable for battery (12V) operation, and also provide closely defined outputs across narrow spectral ranges enabling responses to colour to be investigated more effectively (Bishop *et al*., 2004a). Light trapping of *C. brevitarsis*, a vector species of the BTV in Australia, was more efficient when incandescent globes were replaced with Green LEDs, and the catches also increased as the intensity of LEDs was increased (Bishop *et al*., 2004b; 2006).

During surveys light traps are usually placed as close to livestock as practically possible. The heights at which traps are operated are in many instances determined by the availability of suitable structures near livestock (Goffredo & Meiswinkel, 2004). The effect of trap height on the numbers of *Culicoides* midges collected has to be evaluated to determine optimal height at which midges are most active when seeking host animals. It remains to be determined to what effect environmental factors, *e.g.* the presence of nearby structures, other light sources and livestock near the trap, might have on the height at which *Culicoides* midges will be active.

It is well established that the presence of livestock in the vicinity of a light trap will increase the numbers of certain species of biting midges to be collected (Bellis & Reid, 1996; Garcia-Saenz *et al*., 2011; Venter *et al*., 2011). The distance over which the light trap will attract midges is not known nor is the distance at which the trap will reflect the midge population. The effect that trap distance from the host animals has on the numbers of *Culicoides* collected has to be evaluated to determine the effect on the number and species composition of the midges collected.

In the present study an effort was made to determine to what extent some of these factors would influence the efficiency of light traps. The newly developed FS trap, described in Chapter 2, was used to determine the influence of trap colour, height and different distances from animals on the numbers and species of *Culicoides* collected.

3.2 Material & Methods

3.2.1 The effect of light source colour on the numbers of *Culicoides* midges collected

Five light source colours, dark blue, green, red, white and yellow were compared. In each of five FS 220V traps the black light tube was replaced by a string of 100 clear LED lights in a clear Perspex casing (Fig. 3.2.1). The various colours were obtained by using different coloured filters inside the casing. As a control the OP 220V trap was included in this comparison.

Figure 3.2.1 The FS trap fitted with a Perspex casing and Green filter

Comparisons were done at the ARC-Onderstepoort Veterinary Institute (25˚83’90”S: 28˚81’10”E; 1219 m above sea level). Traps with different coloured light sources were deployed in two replicates of a 6 x 6 randomized Latin square design (Snedecor & Cochran, 1980) to avoid the effect of trap site and night interactions (Perry *et al*., 1980). The traps were compared on 12 consecutive nights during April and May 2008. Traps were operated from dusk to dawn at opposite ends of open-sided barns housing between 20 and 40 cattle. They were hung 1.4 m above ground level and as close to

the cattle as practically possible. To prevent interference between traps all were located at least 15 m apart.

3.2.2 The effect of light trap height on the numbers of *Culicoides* midges collected

FS 12V traps, fitted with 8W black light tubes, were used as some locations did not have any 220V power source. Trap efficiency was evaluated at 1.5 m, 2.0 m, 2.5 m and 3.0 m above ground level. They were placed on poles planted at the different sites. The traps were compared on 12 nights from 13 to 29 January 2009 in three replicates of a 4 x 4 randomized Latin square design (Snedecor & Cochran, 1980). They were operated from dusk to dawn near stables housing six horses approximately 15 km outside Bloemfontein. To avoid interaction between traps, they were placed in such a manner as not to be within direct sight of each other. The traps were not put up as close as possible to host animals as they would have influenced each other being closer than ten metres apart. Two of the trapping sites were therefore further (< 30 m) from the host animals. The randomising of the height treatments in a Latin square design would, however, minimise the impact of site differences on the results.

3.2.3 The effect of distance of host animals from the light source on the numbers of *Culicoides* midges collected

FS 12V traps, fitted with 8W black light tubes, were used as there was no access to a 220V power source. Traps were operated 0 m, 5 m, 15 m and 30 m from the animals at four sites each, two near horses and two near sheep. The comparisons were done on 12 nights from 02 to 19 February 2009 in three replicates of a 4 x 4 randomized Latin square design (Snedecor & Cochran, 1980). Traps were operated from dusk to dawn at stables housing six horses and also at a flock of 35 sheep approximately 15 km outside Bloemfontein.

On all trial nights when no or very few *Culicoides* midges were collected, due to adverse weather conditions or trap failure, collections were repeated the following night. The collection procedure, species analyses and statistical analyses were done as described in Chapter 2.

3.3 Results

3.3.1 The effect of light source colour on the abundance of *Culicoides* midges collected

In 72 collections made 88 801 *Culicoides* of 12 species were collected over 12 nights (Table 3.3.1). Of all the source colours tested, the control OP 220V trap collected significantly more *Culicoides* midges (64.2% of the total) than any of the coloured traps ($P < 0.001$) (Table 3.3.2). The trap fitted with a yellow light collected the lowest number (4.0 % of the total catch) (Table 3.3.2). The low numbers collected with the yellow light was only significantly different ($P = 0.045$) from the white and the OP traps ($P < 0.001$). The average numbers of *Culicoides* collected with the other LEDs did not differ significantly from each other (Table 3.3.2). The percentage (compared to the OP trap) collected ranged from 6.3% with the yellow to 21.2% with white light when compared to the OP trap (Table 3.3.2). The ratio of other insects collected to the number of *Culicoides* ranged from 1:2 with yellow light to 1:4 with the OP trap and red light (Table 3.3.2).

Only six (*C. imicola, C. bedfordi, C. magnus, C. enderleini, C. leucostictus* and *C. zuluensis*) of 12 species collected were present in all the treatments (Table 3.3.1). The traps with the green and red light collected seven species each and the OP 220V trap 10 (Table 3.3.1). Differences in species richness between treatments were the result of a single specimen of some species which was collected on only a few trapping occasions (Table 3.3.1). Species diversity ranged from H= 0.24 (Yellow light) to H= 1.73 (UV light) (Table 3.3.1).

In all six treatments *C. imicola* was the most abundant species, ranging from 95.7% in the yellow light trap to 98.2% in the blue and white traps (Table 3.3.2). The yellow attracted a significantly smaller proportion of *C. imicola* than the blue ($P = 0.041$) and the white light ($P = 0.030$). The second most abundant in all six different trapping regimes was *C. bedfordi*. Its abundance ranged from 0.4% in the green to 1.4% in the yellow light trap. No significant differences were observed in the proportion of *C. bedfordi* collected to that of other species between the different traps.

In addition to the differences in species composition, relatively small but significant differences were found in the age grading and sex ratios with the different traps. The

proportion nulliparous *C. imicola* females ranged from 53.7% (white light) to 62.6% (green light). The white light proportionally attracted significantly fewer nulliparous *C. imicola* females than the OP 220V ($P < 0.001$), green light ($P = 0.003$), red ($P = 0.007$) and yellow ($P = 0.018$). The green light attracted a significantly greater proportion nulliparous *C. imicola* than the blue ($P = 0.046$). The percentage parous *C. imicola* females ranging from 35.5% (green light) to 44.0% (white light). The proportion of parous females collected with the blue light (43.2%) was significantly higher than with the OP trap (36.0%) ($P = 0.084$) and the green light ($P = 0.023$) (Table 3.3.2). The white light (44.0%) collected a significantly greater proportion of parous females than the OP trap (36.0%) ($P < 0.001$), green ($P = 0.004$), red ($P = 0.026$) and the yellow light ($P = 0.025$). No other differences were considered statistically significant. Freshly blood-fed and gravid *C. imicola* females each formed < 0.5% of the total number collected (Table 3.3.2) and no significant differences were found between treatments ($P > 0.05$). The proportion of males collected with the OP trap (3.4%) was significantly higher than males collected in the blue and red light ($P = 0.002$ in both cases). The proportion of males collected with the white light (2.1%) was significantly higher than the red light ($P = 0.022$).

In the second most abundant species, *C. bedfordi*, no significant differences were recorded between any of the colours.

Table 3.3.1 A summary of all *Culicoides* species collected with different coloured light traps at ARC-OVI during April and May 2008

Trap	OP-UV		Blue		Green		Red		White		Yellow	
	Frequency in 12 collections	Total Collected (%)	Frequency in 12 collections	Total Collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)
C. imicola	12	55 510 (97.4)	12	6 075 (98.2)	12	4 598 (96.9)	12	5 108 (97.7)	12	11 835 (98.2)	12	3439 (95.7)
C. bedfordi	11	610 (1.1)	5	30 (0.5)	5	21 (0.4)	5	28 (0.5)	3	68 (0.6)	6	49 (1.4)
C. magnus	7	250 (0.4)	9	55 (0.9)	9	67 (1.4)	8	49 (0.9)	7	64 (0.5)	11	64 (1.8)
C. enderleini	8	320 (0.6)	5	17 (0.3)	6	35 (0.7)	5	27 (0.5)	6	68 (0.6)	8	23 (0.6)
C. leucostictus	3	130 (0.2)	1	4 (<0.1)	3	6 (0.1)	2	2 (<0.1)	3	10 (<0.1)	3	8 (0.2)
C. zuluensis	3	60 (0.1)	1	2 (<0.1)	7	14 (0.3)	4	13 (0.2)	2	6 (<0.1)	1	1
C. nivosus	2	40 (<0.1)	1	1 (<0.1)			2	2 (<0.1)	3	6 (<0.1)		
C. pycnostictus	2	30 (<0.1)	1	2 (<0.1)	1	2 (<0.1)			1	1 (<0.1)		
C. similis	1	20 (<0.1)									1	6 (0.2)
C. expectator	1	20 (<0.1)									1	2 (<0.1)
C. neavei			1	2 (<0.1)								
C. bolitinos											1	1 (<0.1)
Species diversity	1.73		1.49		1.48		1.53		1.56		0.24	
Total	56990		6188		4743		5229		12058		3593	
(% of total collected)	(64.2)		(7.0)		(5.3)		(5.9)		(13.6)		(4.0)	

Table 3.3.2 A summary of *Culicoides* midges collected with different coloured light traps at ARC-OVI during April and May 2008.

Trap	**OP 220V**	**Blue**	**Green**	**Red**	**White**	**Yellow**
No. of collections made	12	12	12	12	12	12
No. of species collected	10	9	7	7	8	9
Total *Culicoides* collected (%)	56990 (64.2)	6188 (7.0)	4743 (5.3)	5229 (5.9)	12058 (13.6)	3593 (4.0)
Mean collection size	4749.17 a	515.67b,c	395.25b,c	435.75b,c	1004.83 b	299.42 c
Range in collection size	930-10840	39-1672	28-1016	32-1565	68-3190	26-990
Comparison with OP 220V trap	100%	10.9%	8.3%	9.2%	21.2%	6.3%
Non-*Culicoides:Culicoides*	1:4	1:3	1:3	1:4	1:3	1:2
Age grading results						
C. imicola						
Mean collected (%)	4 625.8 (97.4)	506.3 (98.2)	383.2 (96.9)	425.7 (97.7)	986.3 (98.2)	286.6 (95.7)
Mean Nulliparous (%)	2770.8 (59.9)	282.5 (55.8)	240.0 (62.6)	262.3 (61.6)	529.8 (53.7)	177.3 (61.9)
Mean Parous (%)	1667.5 (36.0)	218.6 (43.2)	135.8 (35.5)	160.2 (37.6)	434.0 (44.0)	104.6 (36.5)
Mean Freshly blood fed (%)	13.3 (0.3)	0.2 (0.0)	0.9 (0.2)	0.8 (0.2)	1.7 (0.2)	0.7 (0.2)
Mean Gravid (%)	18.3 (0.4)	0.8 (0.1)	0.9 (0.2)	0.2 (0.0)	0.3 (0.0)	0.3 (0.1)
Mean Males (%)	155.8 (3.4)	4.3 (0.8)	5.5 (1.4)	2.2 (0.5)	20.6 (2.1)	3.7 (1.3)
C. bedfordi						
Mean collected (%)	50.8 (1.1)	2.5 (0.5)	1.8 (0.4)	2.3 (0.5)	5.7 (0.6)	4.1 (1.4)
Mean Nulliparous (%)	23.3 (45.9)	1.1 (43.3)	0.8 (42.9)	1.3 (53.6)	3.2 (55.9)	1.8 (42.9)
Mean Parous (%)	14.2 (27.9)	0.8 (30.0)	0.9 (52.4)	0.8 (35.7)	2.2 (38.2)	2.2 (53.1)
Mean Freshly blood fed (%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean Gravid (%)	1.7 (3.3)	0.0 (0.0)	0.1 (4.8)	0.0 (0.0)	0.0 (0.0)	0.2 (4.1)
Mean Males (%)	11.7 (23.0)	0.7 (26.7)	0.0 (0.0)	0.3 (10.7)	0.3 (5.9)	0.0 (0.0)

Significant differences between traps are indicated with a letter (a, b & c) next to the average numbers.

3.3.2 Influence of trap height

In 48 collections made 37 773 *Culicoides* midges belonging to 13 species were collected during 12 nights from 13 to 29 January 2009. Of all the heights evaluated, the traps at 2 m collected the most *Culicoides* midges (27.2% of the total) (Table 3.3.4). The traps at 1.5 m collected the lowest number (19.9%) (Table 3.3.4). These differences were not statistically significant ($P = 0.445$).

Nine of the 13 species collected were present at all the trapping heights namely: *C. leucostictus, C. pycnostictus, C. imicola, C. nivosus, C. similis, C. bedfordi, C. bolitinos, C. neavei* and *C. exspectator*. The traps at 1.5 m and 3 m collected up to ten species each and the traps put up at 2.5 m collected 12 of the 13 species (Table 3.3.3). The species diversity ranged from H = 1.16 (1.5 m) to H = 1.41 (3.0 m) (Table 3.3.3).

At all four trapping heights, *C. leucostictus*, ranging from 40.5% (3 m) to 55.1% (1.5 m) was the most abundant species. The second most abundant was *C. pycnostictus*, ranging from 29.0% at 2 m to 37.0% at 2.5 m (Table 3.3.3). No significant differences were observed in either the average numbers of *C. leucostictus* or that of *C. pycnostictus* between the different heights ($P = 0.726$).

Culicoides imicola was the third most abundant species to be collected and its abundance ranged from 5.7% (1.5 m) to 10.8% (3 m) (Table 3.3.4). In contrast to the previous two species significantly more *C. imicola* were collected at 3 m (mean 90.6) than at 1.5 m (mean 36.0) ($P = 0.004$).

Culicoides leucostictus males were the dominant gender to be collected (Table 3.3.4). The proportion of males collected ranged from 67.5% (3 m) to 74.8% (1.5 m) (Table 3.3.4), and this was considered significant ($P = 0.036$). Significantly fewer males were collected at 3.0 m than at 2.0 m ($P = 0.049$). The percentage nulliparous *C. leucostictus* females ranged from 6.4% (1.5 m) to 17.1% (3 m). The traps placed at 3.0 m collected a significantly higher proportion of nulliparous female *C. leucostictus* than the traps at 2.0 m ($P = 0.004$) or 1.5 m ($P < 0.001$). A significantly higher proportion of nulliparous midges were collected at 2.5 m than at 1.5 m ($P = 0.003$). Significantly more parous *C. leucostictus* were collected at 1.5 m than at 2.5 m ($P = 0.045$). No significant differences in the proportion freshly blood-fed or gravid females were recorded at the different trapping heights (Table 3.3.4).

Similar to *C. leucostictus*, males were also the dominant gender to be collected of *C. pycnostictus* (Tabel 3.3.4). The proportion of male *C. pycnostictus* collected at the different trapping heights was not significantly different.

The results obtained for *C. pycnostictus* showed that a significantly higher proportion nulliparous females were collected at 3.0 m than at 1.5 m ($P = 0.012$), 2.0 m ($P = 0.005$) and 2.5 m ($P < 0.001$). A significantly smaller proportion parous *C. pycnostictus* were collected at 3.0 m than at 1.5 m ($P = 0.023$) and 2.5 m ($P = 0.009$). No significant differences were observed in the proportion freshly blood-fed *C. pycnostictus* collected at the different trapping heights. A significantly greater proportion gravid females were collected at 2.0 m than at 2.5 m ($P = 0.001$) and 3.0 m ($P = 0.001$).

A significantly greater proportion nulliparous *C. imicola* females were collected at 2.0 m than at 3.0 m ($P = 0.045$). No significant differences in the proportion of parous, freshly blood-fed or gravid female *C. imicola* collected at the different heights were observed. In contrast to *C. leucostictus* and *C. pycnostictus*, males were not the dominant gender to be collected. The percentage male *C. imicola* ranged from 7.4% at 2.5 m to 21.5% at 3.0 m (Table 3.3.4). Significantly more males were collected at 3.0 m than at 2.5 m ($P = 0.006$).

3.3.3 Efficacy of traps at different distances from host animals

In 48 collections made 18 084 *Culicoides* midges belonging to 14 species were collected over 12 nights from 2 to 19 February 2009 (Table 3.3.5). Of all the distances evaluated, the traps closest to the animals (0 m) collected the highest number of *Culicoides* (39.6% of the total) (Table 3.3.6). The traps furthest from livestock (30 m) collected the lowest number of midges (10.5% of the total catch) (Fig. 3.3.6). The traps closest to the hosts (0 m) collected significantly more *Culicoides* midges than the traps 15 m ($P = 0.035$) and 30 m ($P = 0.001$) from the animals. The traps collected significantly more midges 5 m from the animals than 30 m ($P = 0.026$).

Table 3.3.3 A summary of all the *Culicoides* species collected with the FS 12 V light trap at different heights near Bloemfontein from 13 January to 29 January 2009.

Trap	1.5m		2m		2.5m		3m		Total collected in all four traps
	Frequency in 12 collections	Total Collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	
C. leucostictus	12	4139 (55.1)	12	5549 (54)	12	4153 (42.1)	12	4091 (40.5)	17932 (47.5)
C. pycnostictus	12	2363 (31.4)	11	2980 (29)	12	3656 (37)	12	3418 (33.8)	12417 (32.9)
C. imicola	11	432 (5.8)	11	951 (9.2)	12	1033 (10.5)	12	1087 (10.8)	3503 (9.3)
C. nivosus	7	280 (3.7)	12	524 (5.1)	12	823 (8.3)	12	1097 (10.9)	2724 (7.2)
C. similis	7	181 (2.4)	4	106 (1)	3	21 (0.2)	8	133 (1.2)	441 (1.2)
C. bedfordi	2	60 (0.8)	4	50 (0.5)	6	48 (0.5)	4	115 (1.1)	273 (0.7)
C. bolitinos	1	20 (0.3)	5	25 (0.2)	3	53 (0.5)	5	88 (0.9)	186 (0.5)
C. neavei	2	10 (0.1)	2	15 (0.2)	4	27 (0.3)	3	61 (0.6)	113 (0.3)
C. exspectator	1	10 (0.1)	4	52 (0.5)	2	30 (0.3)	1	20 (0.2)	112 (0.3)
C. zuluensis			3	12 (0.1)	2	12 (0.1)	1	1 (<0.1)	25 (<0.1)
C. enderleini	1	20 (0.3)							20 (<0.1)
C. magnus			1	5 (<0.1)	1	10 (0.1)			15 (<0.1)
C. albopunctatus					1	10 (0.1)			10 (<0.1)
Species diversity	1.16		1.20		1.30		1.41		
Total (% of total collected)		7515 (19.90)		10269 (27.19)		9878 (26.15)		10111 (26.77)	37773

Table 3.3.4 A summary of *Culicoides* midges collected with the FS 12 V light trap at different heights near Bloemfontein from 13 January to 29 January 2009.

Trap	**1.5m**		**2m**		**2.5m**		**3m**	
No. of collections made	12		12		12		12	
No. of species collected	10		11		12		10	
Total *Culicoides* collected (%)	7515	(19.90)	10269	(27.19)	9878	(26.15)	10111	(26.77)
Mean collection size	626.25		855.75		823.17		842.58	
Range in collection size	12-3500		3-4140		40-2700		109-3280	
Age grading results								
C. leucostictus								
Mean collected (%)	344.9	(55.1)	462.4	(54.0)	346.1	(42.0)	340.9	(40.5)
Mean Nulliparous (%)	21.9	(6.4)	46.3	(10.0)	45.8	(13.2)	58.4	(17.1)
Mean Parous (%)	49.1	(14.2)	54.8	(11.8)	32.0	(9.2)	37.1	(10.9)
Mean Freshly blood fed (%)	0.0	(0.0)	0.8	(0.2)	1.7	(0.5)	0.0	(0.0)
Mean Gravid (%)	16.0	(4.6)	18.3	(3.9)	11.0	(3.2)	15.4	(4.5)
Mean Males (%)	257.9	(74.8)	342.3	(74.0)	255.6	(73.9)	230.0	(67.5)
C. pycnostictus								
Mean collected (%)	196.9	(31.4)	248.3	(29.0)	304.7	(37.0)	284.8	(33.8)
Mean Nulliparous (%)	33.7	(17.1)	42.2	(17.0)	41.6	(13.6)	77.8	(27.3)
Mean Parous (%)	50.5	(25.6)	50.6	(20.4)	79.8	(26.2)	48.5	(17.0)
Mean Freshly blood fed (%)	1.7	(0.8)	0.4	(0.2)	0.0	(0.0)	1.9	(0.7)
Mean Gravid (%)	28.3	(14.3)	46.4	(18.7)	26.8	(8.8)	24.8	(8.7)
Mean Males (%)	82.8	(42.1)	108.8	(43.8)	156.4	(51.3)	131.8	(46.3)
C. imicola								
Mean collected (%)	36.0	(5.7)	79.3	(9.3)	86.1	(10.5)	90.6	(10.8)
Mean Nulliparous (%)	19.3	(53.5)	42.6	(53.7)	42.0	(48.8)	34.3	(37.8)
Mean Parous (%)	10.4	(28.9)	20.5	(25.9)	34.0	(39.5)	27.8	(30.7)
Mean Freshly blood fed (%)	0.0	(0.0)	0.2	(0.2)	0.0	(0.0)	0.0	(0.0)
Mean Gravid (%)	0.1	(0.2)	3.7	(4.6)	3.7	(4.3)	9.0	(9.9)
Mean Males (%)	6.3	(17.4)	12.3	(15.6)	6.3	(7.4)	19.5	(21.5)

Nine of the 14 species collected were present at all the trapping distances namely: *C. imicola, C. leucostictus, C. pycnostictus, C. nivosus, C. bedfordi, C. bolitinos, C. similis, C. exspectator* and *C. zuluensis*. The closest (0 and 5 m) to the host collected 10 species each and the traps put up further (15 and 30 m) collected 12 species each (Table 3.3.5). The species diversity range was from H = 0.88 (0 m) to H = 1.61 (30 m) (Table 3.3.5)

The mean number of *C. imicola* collected dropped from 455.6 at 0 m to 28.9 at 30 m away from livestock (Figure 3.3.1). *Culicoides imicola* was the dominat species to be collected up to 15 m from the host (Tabel 3.3.6). Its abundance dropped from 76.4% at 0 m to 30.2% at 15 m (Tabel 3.3.6). At 30 m it represented only 18.3% of all species collected and was only the third most abundant species after *C. pycnostictus* (35.9%) and *C. leucostictus* (28.2%) (Table 3.3.5). The proportion of *C. imicola* of the total collected decreased significantly when compared to 5 m ($P = 0.001$), 15 m ($P < 0.001$) and 30 m ($P < 0.001$) from the livestock.

The second most abundant *Culicoides* species was *C. leucostictus*. Its abundance ranged from 10.7% at 0 m to 29.8% at 15 m. Different from *C. imicola* the abundance of *C. leucostictus* did not drop in relation to the distance from livestock (Figure 3.3.6). On average it was the most abundant at 15 m (118.2), then at 5 m (79.8), 0 m (63.5) and it was the least abundant at 30 m (44.7) (Figure 3.3.6). The proportion of *C. leucostictus* to total *Culicoides* collected right next to the livestock increased significantly when compared to 5 m ($P = 0.023$), 15 m ($P < 0.001$) and 30 m ($P < 0.001$) from the hosts. When comparing the average *C. leucostictus* collected at the 5 m, 15 m and 30 m sites, no significant differences were observed ($P = 0.254$).

No significant differences were found in the proportion nulliparous or freshly blood-fed *C. imicola* collected at the different trapping distances. The proportion gravid *C. imicola* was significantly less at 0 m ($P= 0.001$), 5 m ($P = 0.007$) and 15 m ($P = 0.013$) compared to 30 m. The percentage male *C. imicola* midges ranged from 4.3% at 0 m to 18.2% at 30 m (Table 3.3.6). A significantly higher proportion of male *C. imicola* were collected at 5 m ($P = 0.002$) 15 m ($P = 0.002$) and 30 m ($P = 0.012$) than right next to the host animals (0 m).

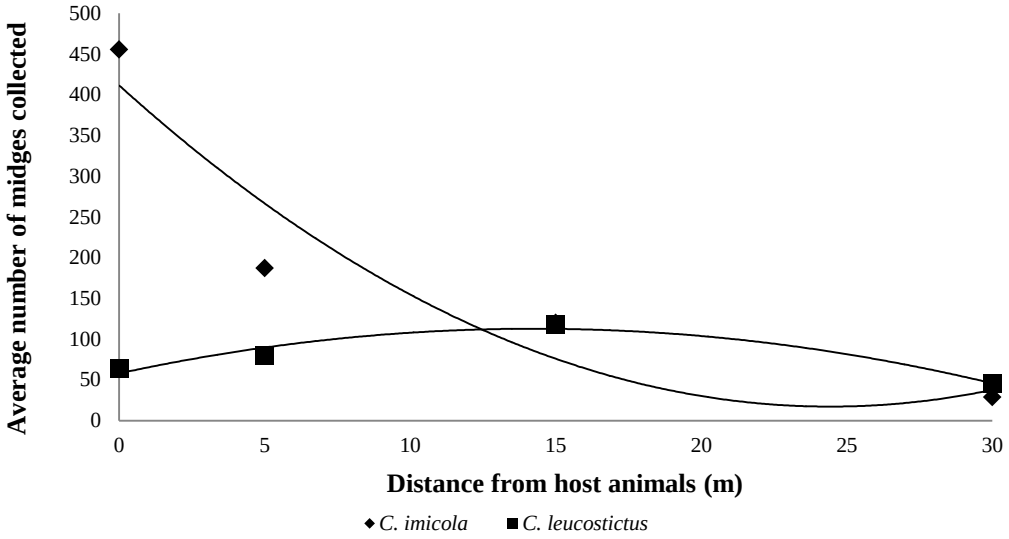

Figure 3.3.1 The mean numbers *C. imicola* and *C. leucostictus* collected with the FS trap at 0, 5, 15 and 30 m from the host animals. A total of 12 collections were done at each distance during February 2009 in Bloemfontein

A significantly smaller proportion nulliparous *C. leucostictus* were collected at 15 m than at 0 m ($P = 0.004$) and 5 m ($P < 0.001$). A significantly smaller proportion of parous *C. leucostictus* were collected at 15 m than at 5 m ($P = 0.013$) (Table 3.3.6). No differences in the proportion gravid *C. leucostictus* were observed between the different distances. A significantly higher proportion of male *C. leucostictus* were collected at 15 m than at 0 m ($P < 0.001$), 5 m ($P < 0.001$) and 30 m ($P < 0.001$) from the hosts.

Table 3.3.5 A summary of all the *Culicoides* species collected with the FS 12 V light trap at different distances from host animals near Bloemfontein from 02 February to 19 March 2009.

Trap distance from animals	0m		5m		15m		30m		Total collected in all four traps
	Frequency in 12 collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	Frequency in 12 Collections	Total collected (%)	Frequency in 12 collections	Total collected (%)	
C. imicola	12	5467 (76.4)	12	2247 (52.7)	12	1440 (30.2)	9	347 (18.3)	9501 (52.5)
C. leucostictus	12	762 (10.7)	11	957 (22.4)	12	1418 (29.8)	12	536 (28.2)	3673 (20.3)
C. pycnostictus	12	485 (6.8)	12	551 (12.9)	12	1264 (26.5)	12	683 (35.9)	2983 (16.5)
C. nivosus	10	187 (2.6)	11	205 (4.8)	12	368 (7.7)	11	179 (9.4)	939 (5.2)
C. bedfordi	9	105 (1.5)	6	121 (2.8)	6	96 (2.0)	6	48 (2.5)	370 (2.0)
C. bolitinos	6	106 (1.5)	9	118 (2.8)	4	22 (0.5)	5	17 (0.9)	263 (1.5)
C. similis	4	30 (0.4)	1	10 (0.2)	4	91 (1.9)	3	26 (1.4)	157 (0.9)
C. neavei			1	10 (0.2)	3	21 (0.4)	5	28 (1.5)	59 (0.3)
C. exspectator	1	2 (<0.1)	3	21 (0.5)	3	18 (0.4)	4	13 (0.7)	54 (0.3)
C. zuluensis	2	6 (0.1)	3	26 (0.6)	2	15 (0.3)	1	1 (0.1)	48 (0.3)
C. magnus							3	15 (0.8)	15 (0.1)
C. enderleini	1	2 (<0.1)					2	9 (0.4)	11 (0.1)
C. dutoiti					1	10 (0.2)			10 (0.1)
C. coarctatus					1	1 (<0.1)			1 (<0.1)
Species diversity	0.88		1.37		1.53		1.61		
Total (% of total collected)	7152 (39.6)		4266 (23.6)		4764 (26.3)		1900 (10.5)		18084

Table 3.3.6 A summary of *Culicoides* midges collected with the FS 12 V light trap at different distances from host animals near Bloemfontein from 02 February to 19 March 2009.

Trap	**0m**	**5m**	**15m**	**30m**
No. of collections made	12	12	12	12
No. of species collected	10	10	12	12
Total *Culicoides* collected (%)	7152 (39.6)	4266 (23.6)	4764 (26.3)	1902 (10.5)
Average collection size	596.00	355.50	397.00	158.33
Range in collection size	140-1230	40-835	9-2460	9-555
Age grading results				
C. imicola				
Mean collected (%)	455.6 (76.4)	187.3 (52.7)	120.0 (30.2)	28.9 (18.3)
Mean Nulliparous (%)	197.4 (43.3)	81.7 (43.6)	57.0 (47.5)	8.9 (30.7)
Mean Parous (%)	232.3 (51.0)	81.3 (43.4)	44.7 (37.2)	10.4 (35.9)
Mean Freshly blood fed (%)	1.3 (0.3)	0.9 (0.5)	1.7 (1.4)	0.1 (0.3)
Mean Gravid (%)	5.0 (1.1)	2.5 (1.3)	1.7 (1.4)	4.3 (15.0)
Mean Males (%)	19.6 (4.3)	20.9 (11.2)	15.0 (12.5)	5.3 (18.2)
C. leucostictus				
Mean collected (%)	63.5 (10.7)	79.8 (22.4)	118.2 (29.8)	44.7 (28.2)
Mean Nulliparous (%)	15.7 (24.7)	25.5 (32.0)	10.0 (8.5)	9.3 (20.7)
Mean Parous (%)	12.2 (19.2)	18.1 (22.7)	10.7 (9.0)	7.9 (17.6)
Mean Freshly blood fed (%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean Gravid (%)	4.7 (7.3)	3.7 (4.6)	4.5 (3.8)	5.7 (12.7)
Mean Males (%)	31.0 (48.8)	32.5 (40.8)	93.0 (78.7)	21.8 (48.9)

3.4 Discussion

3.4.1 Influence of trap colour for *Culicoides* attraction

Culicoides midges were attracted to all of the colours evaluated and all traps collected *Culicoides* midges each night they were operated. At all traps *C. imicola* was found to be the most abundant species and *C. bedfordi* the second most abundant. The control OP 220V light trap was the most efficient light trap when collecting *Culicoides* midges.

There were no clear distinctions of species preference for any of the different light colours. There were no significant differences in total *Culicoides* collected between the blue, green, red and yellow coloured lights. The differences in attraction of species to colours as observed by Bishop *et al.* (2006) in Australia were not seen in this study. The species collected were not the same as those collected by Bishop *et al.* (2006), however, and this may be the crucial difference. The fact that more midges were collected in the traps fitted with white light (no filters) leads to the conclusion that intensity may have played a much greater role than colour. The UV light fitted in the OP trap was far brighter and the white light without any filter was also brighter than those with coloured filters. In future research this is an important criterion to be standardized in order to evaluate the differential effect of trap colour more clearly.

The age grading of the midges also did not differ significantly as all colours attracted mostly nulliparous *Culicoides* females, varying from 54.9% by the white light to 63.4% by the green filter (Table 3.3.2). Of the midges collected by the different lights these all attracted less than 1% freshly blood-fed and gravid females. These results confirm that light traps, irrespective of light colour, predominantly collect female *Culicoides* which are actively seeking a blood meal.

3.4.2 Influence of trap height

All trap heights evaluated collected *Culicoides* midges on each night they were operated. At all heights *C. leucostictus* was the most abundant species and *C. pycnostictus* as the second most abundant species. These were definitely influenced by the trapping sites as they were located in wooded areas, which may account for the

dominance of the bird-feeding species. The relatively high percentage of males in the collections may indicate that the traps were operated near a potential breeding site.

Differing from the previous two, significantly more *C. imicola* were collected at 3 m (mean 90.6) than at 1.5 m (mean 36.0) (P = 0.004). The largest numbers were collected in the traps 3m above ground level. The number of *C. imicola* doubled when collected 1.5 m to 2.0 m or higher and *C. imicola* also occurred in all the collections above 2.0 m. There were no significant differences between the numbers collected at 2.0 m to 3.0 m. This indicates that *C. imicola* is more active higher than 2.0 m when seeking a suitable host. Venter *et al*. (2009) also found than *C. imicola* was more abundant when collecting higher, but also found differences between each height. These results again indicated that *C. imicola* may have a preferred height for host seeking but the effect of the nearby structures and trees also needs to be evaluated. It is worth mentioning that Braverman and Linley (1993) were able to collect *C. imicola* 26 m above ground level. In contrast to *C. imicola*, the trapping height did not have a significant influence on *C. pycnostictus* and *C. leucostictus*. This indicates species specificity for flying height that should be evaluated further. The trapping height is definitely another variable that has to be standardized. Since the presence of hosts and other environmental factors can be an influence, it will be difficult to standarize this particular variable.

3.4.3 Efficacy of traps at different distances from host animals

All trapping distances collected *Culicoides* midges on each night. From 0 m up to 15 m from livestock *C. imicola* was the dominant species and at 30 m *C. pycnostictus* was the dominant one.

The light trap distance from animals proved to be an important variable when collecting and monitoring *Culicoides* midges. Traps operated immediately next to the host, collected the most *Culicoides* midges. This in itself sounds obvious but when compared to collections made only 5 m away, the numbers of species dropped by almost a third. The percentage male *C. imicola* increased as the collections moved farther away but those numbers also decreased. The percentage gravid females gradually increased moving farther and that indicated possible preference for breeding sites farther away.

The distance from livestock collections highlighted the host preference of *C. imicola* for sheep and horses, and also the relative abundance of *C. leucostictus*, a known bird-feeding species, which stayed seemingly constant throughout the collections.

To optimize midge trapping various methods have been evaluated and Mands *et al*. (2004) evaluated the enhancement of traps with animal odour. They had the greatest success with water buffalo extracts as it increased the numbers of the catches by 262%. Cilek & Kline (2002) used baited traps with a combination of octenol-phenol and CO_2 and collected significantly more midges than without any bait. This indicates the attraction of the animal odour plume rather than light when collecting *Culicoides* midges. It even showed that the light traps do not attract midges from farther than 5 m. The use of chemical attractants (Kline *et al*., 1994; Ritchie *et al*., 1994; Braverman *et al*., 2000; Cilek & Kline, 2002) may well increase the efficiency of these traps for *Culicoides* midges.

Light trap design is constantly changing in an effort to improve quality and specificity. Light traps are exposed to harsh conditions and breakages need to be minimized so as not to interrupt monitoring.

Suction light traps have proved to be a very effective and practical method to sample midge populations (Venter *et al.*, 1996; 2006). It must, however, be accepted that a great number of environmental factors can influence the results obtained with light traps. Climatic factors such as the temperature and wind speed (Murray, 1987; Edwards *et al*., 1987), rainfall and relative humidity (Reuben, 1963) can all have an effect on the numbers of *Culicoides* collected. Several factors, other than weather conditions, influence the number. These factors include the presence of breeding sites, other light sources, the height of the trap above ground level (Murray, 1987; Venter *et al*., 2009a) and even the phase of the moon (Nelson & Bellamy, 1971; Barnard & Jones, 1980; Edwards *et al.*, 1987). The results of this study showed that despite a variety of factors that can influence the numbers caught, traps are still a practical and reliable way to determine presence and abundance of midges in a given area. In interpreting light trap results the limitations of this collection method and the factors that can influence the results need to be taken into consideration. Factors that contribute to variability in trap data will make it difficult for reliable comparisons of data between different locations and countries to be made. This highlights the need for

standardized trapping methods to be developed and adopted when using light traps to determine the vectorial capacity of *Culicoides* midges.

3.5. References

Barnard, D.R. & Jones, R.H. 1980. *Culicoides variipennis* seasonal abundance, overwintering, and voltinism in northeastern Colorado. *Environmental Entomology*, **9**, 709-712.

Bellis, G.A. & Reid, D.J. 1996. Sampling bias in determining the parous rate of collections of *Culicoides brevitarsis* Kieffer and *C. wadai* Kitaoka (Diptera: Ceratopogonidae). *Australian Journal of Entomology*, **35**, 319–322.

Bishop A.L., Bellis, G.A., McKenzie, H.J., Spohr, L.J., Worrall, R.J., Harris, A.M. & Melville, L. 2006. Light trapping of biting midges *Culicoides* spp. (Diptera: Ceratopogonidae) with green light-emitting diodes. *Australian Journal of Entomology*, **45**, 202-205.

Bishop, A.L., Worall, R., Spohr, L.J., McKenzie, H.J. & Barchia, I.M. 2004a. Improving light-trap efficiency for *Culicoides* spp. with light-emitting diodes. *Veterinaria Italiana,* **40**, 266-269.

Bishop, A.L., Worall, R., Spohr, L.J., McKenzie, H.J. & Barchia, I.M. 2004b. Response of *Culicoides* spp. (Diptera: Ceratopogonidae) to light-emitting diodes. *Australian Journal of Entomology,* **43**, 184-188.

Braverman, Y. & Linley, J.R. 1993. Effect of light trap height on catch of *Culicoides* (Diptera: Ceratopogonidae) in Israel. *Journal of Medical Entomology*, **30**, 1060-1063.

Braverman, Y., Wegis, M.C. & Mullens, B.A., 2000. Response of *Culicoides sonorensis* (Diptera: Ceratopogonidae) to 1-octen-3-ol and three plant-derived repellent formulations in the field. *Journal of the American Mosquito Control Association*, **16**, 158-163.

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. & Mellor, P. 2008. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential Bluetongue virus vector? *Journal of Applied Ecology*, **45**, 1237-1245.

Cilek, J.E. & Kline, D.L. 2002. Adult biting midge response to trap type, carbon dioxide, and an octenol-phenol mixture in Northwestern Florida. *Journal of the American Mosquito Control Association*, **18**, 228-231.

Edwards, P.B., Kettle, D.S. & Barnes, A. 1987. Factors affecting the numbers of *Culicoides* (Diptera: Ceratopogonidae) in traps in coastal south-east Queensland, with particular reference to collections of *C. subimmaculatus* in light traps. *Australian Journal of Zoology*, **35**, 469-486.

Garcia-Saenz, A., McCarter, P. & Baylis, M. 2011. The influence of host number on the attraction of biting midges, *Culicoides* spp., to light traps. *Medical and Veterinary Entomology*, **25**, 113-115.

Gerry, A.C., Sarto I Monteys, V., Moreno Vidal, J.O., Francino, O. & Mullens, B.A., 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in northeastern Spain in relation to midge capture using UV light and carbon dioxide-baited traps. *Journal of Medical Entomology*, **46**, 615-624.

Goffredo, M. & Meiswinkel, R. 2004. Entomological surveillance of Bluetongue in Italy: methods of capture, catch analysis and identification of *Culicoides* biting midges. *Veterinaria Italiana*, **40**, 260-265.

Kline, D.L., Hagan, D. & Wood, J.R. 1994. *Culicoides* responses to l-octen-3-ol and carbon dioxide in salt marshes near Sea Island, Georgia, U.S.A. *Medical and Veterinary Entomology*, **8**, 25-30.

Mands, V., Kline, D.L. & Blackwell, A. 2004. *Culicoides* midge trap enhancement with animal odour baits in Scotland. *Medical and Veterinary Entomology*, **18**, 336-342.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: *Infectious Diseases of Livestock* (ed. by J.A.W. Coetzer and R.C. Tustin), pp. 93-136. Oxford University Press, Cape Town.

Mellor, P.S., Tabachnick, W., Baldet, T., Baylis, M., Bellis, G., Calistri, P., Delécolle, J-C., Goffredo, M., Meiswinkel, R., Mullens, B., Scaramozzino, P., Torina, A., Venter, G. & White, D. 2004. Conclusions of working groups. Group 2. Vectors. Proceedings of the 3rd OIE Bluetongue International Symposium, 26–29 October 2003, Taormina, Italy. *Veterinaria Italiana*, **40**, 715–717.

Murray, M.D. 1987. Local dispersal of the biting midge *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in southern Australia. *Australian Journal of Zoology*, **35**, 559-573.

Nelson, R.L. & Bellamy, R.E. 1971. Patterns of flight activity of *Culicoides variipennis* (Coquillett) (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, **8**, 283-291.

Perry, J.N., Wall, C. & Greenway, A.R. 1980. Latin square designs in field experiments involving sex attractants. *Ecological Entomology*, **5**, 385–396.

Reuben, R. 1963. A comparison of trap catches of *Culicoides impunctatus* Goetghebuer (Diptera: Ceratopogonidae) with meteorological data. *Proceedings of the Royal Entomological Society of London*, **38**, 181-193.

Ritchie, S.A., van Essen, P.H.A., Kemme, J.A., Kay, B.A. & Allaway, D. 1994. Response of biting midges (Diptera: Ceratopogonidae) to carbon dioxide, octenol, and light in Southeastern Queensland. *Australian Journal of Medical Entomology*, **31**, 645-648.

Rowley, W.A. & Jorgensen, N.M. 1967. Relative effectiveness of three types of light traps in collecting adult *Culicoides. Journal of Economic Entomology*, **60**, 1478-1479.

Service, M.W. 1977. A critical review of procedures for sampling populations of adult mosquitoes. *Bulletin of Entomological Research*, 67, 343-382.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical Methods, 7th edition. Iowa State University Press.

Venter, G.J. & Hermanides, K.G. 2006. Comparison of black and white light for collecting *Culicoides imicola* and other livestock-associated *Culicoides* species in South Africa. *Veterinary Parasitology,* **142**, 383–385.

Venter, G.J., Herimandes, K.G., Boikanyo, S.N.B., Majatladi, D.M. & Morey, L. 2009a. The effect of light trap height on the numbers of *Culicoides* midges collected under field conditions in South Africa. *Veterinary Parasitology,* **166**, 343-345.

Venter, G.J., Koekemoer, J.J.O. & Paweska, J.T. 2006. Investigations on outbreaks of African horse sickness in the surveillance zone of South Africa. *Revue scientifique et technique, Office International des Epizooties,* **25**, 1 097-1 019.

Venter, G.J., Labuschagne, K., Boikanyo, S.N.B., Matjatladi, D.M. & Morey, L. 2011. The effect of 1-octen-3-ol and 4-methylphenol on *Culicoides* midge numbers collected with suction light traps in South Africa. *Veterinary Parasitology*, **175**, 182–186.

Venter, G.J., Labuschagne, K., Hermanides, K.G., Boikanyo, S.N.B., Majatladi, D.M. & Morey, L. 2009b. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Veterinary Parasitology*, **166**, 299–307.

Venter, G.J., Nevill, E.M. & van der Linde, T.C. 1996. Geographical distribution and relative abundance of stock associated *Culicodes* species (Diptera: Ceratopogonidae) in southern Africa, in relation to their potential as viral vectors. *Onderstepoort Journal of Veterinary Research,* **63**, 25-38.

4. SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF LIVESTOCK ASSOCIATED *CULICOIDES* MIDGES IN THE CENTRAL FREE STATE PROVINCE OF SOUTH AFRICA

4.1 Introduction

From 1955 to 1995 outbreaks of AHS occurred every summer in the northern parts of South Africa from where it spreads to the south (Coetzer & Guthrie, 2004). Depending on the climatic conditions of a specific year outbreaks only occur every five to ten years in the central parts of the country (Coetzer & Guthrie, 2004). Since 1955 outbreaks of AHS are believed to have been relatively rare in the most southern parts of the Western Cape Province (Guthrie, 1999).

Following an unpredicted outbreak of AHS, in which approximately 100 horses died, in the colder eastern parts of Free State Province between February and May 1998 (Meiswinkel & Paweska, 2003), the number of outbreaks also seems to have increased in the central and more southern parts of the country (AHS Trust, 2012). Despite being a notifiable disease in South Africa, outbreaks are relatively poorly documented as horse owners do not readily report this information to the state veterinarian. Notwithstanding the relatively harsh conditions, the Free State is considered as an endemic area for the occurrence of AHS and BT.

In South Africa *Culicoides* midge abundance is closely linked to climatic factors (Meiswinkel *et al*., 2004). Relatively large midge numbers can be collected by light traps throughout the year in the frost free summer rainfall areas (Venter *et al*., 1997; Meiswinkel *et al*., 2004). In these areas breeding is continuous throughout the year and the numbers of *C. imicola* can become exceptionally high in the vicinity of livestock at the end of summer (Venter *et al*., 1997; Meiswinkel *et al*., 2004). Viral transmission can theoretically occur throughout the year creating possible enzootic areas for AHS and BT (Venter *et al*., 1997; Guthrie, 1999; Meiswinkel *et al*., 2004). As the altitude increases towards the central plateau there is a corresponding drop in winter temperatures. In the summer rainfall areas where frost occurs, *Culicoides* midges usually disappear from collections after the first frost of winter (Bosman *et al*., 1995; Venter *et al*., 1997; Meiswinkel *et al*., 2004). As temperatures increase in

spring, *Culicoides* numbers slowly build up after the winter months until they peak in the second half of the summer (Bosman *et al.*, 1995; Venter *et al*., 1997).

Culicoides imicola, the most abundant livestock-associated *Culicoides* species in South Africa, seems to be absent from areas where the average daily maximum temperatures during the coldest months of the year are below 12.5°C (Wittman *et al*., 2001). Midge abundance also seems to be associated with rainfall and can vary according to the amount of rain during a specific time of the year (Baylis *et al*., 1999; Meiswinkel *et al.*, 2004). *Culicoides imicola* tends to occur in areas with an annual rainfall of 300-700 mm (Wittman *et al*., 2001). Factors like irrigated pastures, leaking water pipes and animal drinking troughs also need to be considered.

The Free State is situated in the centre of South Africa and Bloemfontein lies on one of the main connection routes between the north and the south of the country. Although the summers are relatively hot, the winters are also relatively cold with several nights below freezing point. In winter the day temperatures range between 10°C and 20°C. The area can be classified as being fairly dry, with most rain occurring in the summer and early autumn. The 30-year mean yearly rainfall recorded by the South African Weather Service from 1961 to 1990 was 559 mm.

Information regarding the occurrence and seasonal abundance of *Culicoides* midges, especially for the colder winter months, is relatively scarce. No information specific to the central Free State was available and the monitoring done in some parts of the eastern Free State was done more than fifteen years ago (Venter & Sweatman, 1989; Venter & Meiswinkel, 1994; Venter *et al.*, 1996; 1997).

In order to determine the risk of AHS and other viral diseases occurring in the central Free State the *Culicoides* species composition and abundance of livestock-associated midges was determined. The seasonal abundance of the midges in the central Free State, and the presence and duration of midge-free periods in winter, should indicate if AHSV and other orbiviruses can overwinter in adult midges in the area. Finally the influence that climate has on the population of midges was determined.

4.2 Material & Methods

4.2.1 Study area

The survey was conducted near Bloemfontein in the central Free Sate. The natural vegetation consisted of grasslands where the land has not been cultivated with wheat or maize. The central Free State is mostly covered by farmland with a mixture of game, cattle and sheep farmers. This is a summer-rainfall area, where the mean maximum summer temperature is 26°C and the average maximum winter temperature 16°C with night-time temperatures falling to a monthly average of -2°C. The average elevation for Bloemfontein is ~1400 m above sea level.

In order to determine the *Culicoides* species composition and seasonal abundance regular light trap collections were made at six sites in the vicinity of horses and sheep in the Bloemfontein area (Fig. 4.2.1). The distance between sites ranged from approximately 1.5 km (site 1 and 2) to approximately 30 km (site 1 and 5) (Fig. 4.2.1). In most cases two traps, at least 15 m apart and not within direct sight of each other, were operated at a site on a trapping night.

Livestock present at the various collection sites were:

Site 1 had horse stables, housing six horses (29˚03’48’’S, 26˚06’34’’E)

Site 2 was a small holding with a flock of 35 sheep (29˚03’24’’S, 26˚06’57’’E)

Site 3 had horse stables holding approximately 25 horses (29˚04’22’’S, 26˚07’35’’E)

Site 4 had horse stables holding approximately 15 horses (29˚05’35’’S, 26˚08’20’’E)

Site 5 was a horse farm, holding approximately 15 horses at a time (29˚06’24’’S, 26˚19’58’’E)

Site 6 was auction grounds, holding cattle, sheep, and goats in varying numbers (29˚03’57’’S, 26˚08’05’’E).

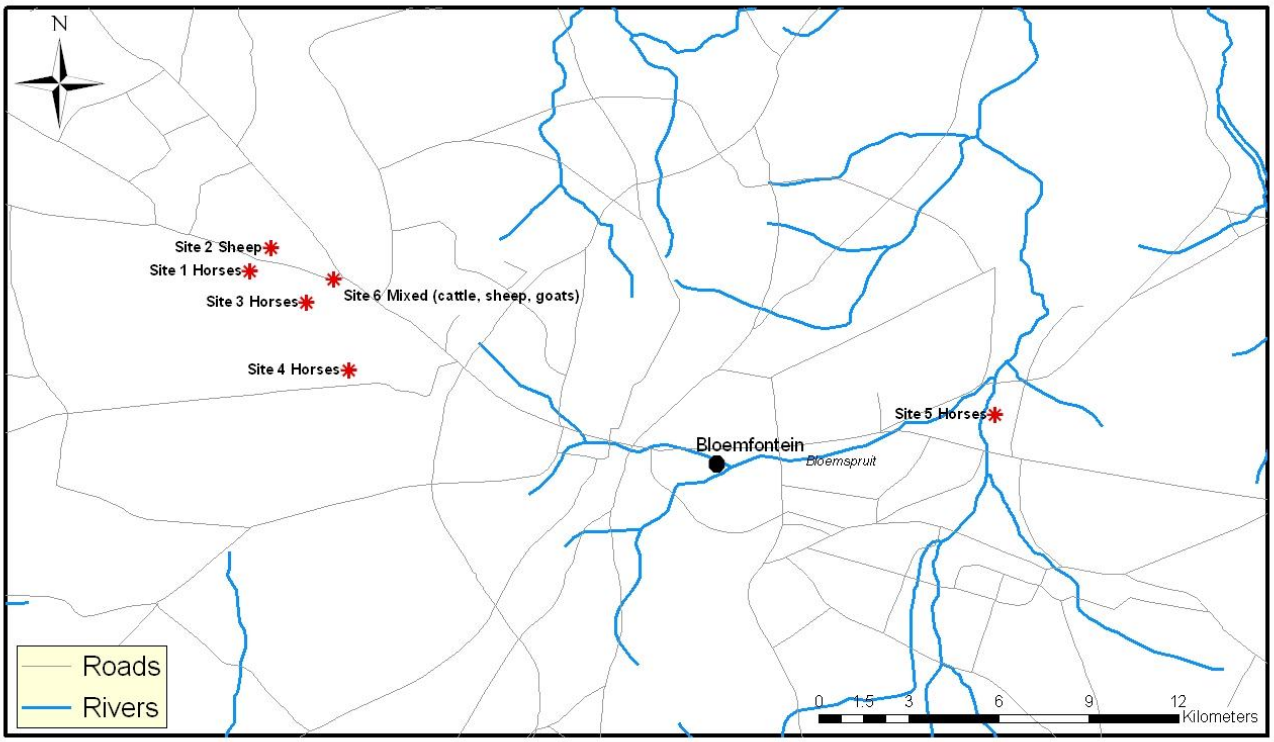

Figure 4.2.1 Distribution of *Culicoides* collection sites around Bloemfontein where FS light traps were operated from February 2007 to May 2010 (Chantel de Beer, ARC-OVI)

4.2.2 Light trap collections

Weekly light trap collections were made at one or more of the collection sites using down-draught FS 220V or 12V light traps equipped with 8W UV light tubes put up near livestock from February 2007 up to May 2010. Light trap collections and analysis of collections were done as described in Chapter 2.2.

Weather data were obtained from the South African Weather Service. The weather station was within five kilometers of the collection sites.

4.3 Results

As a result of accessibility and logistics 220 of the 261 collections were made at Site 1 (n = 187) and 4 (n = 33) (Fig. 4.2.1). The other four sites were only sampled randomly making meaningful comparisons impossible and irrelevant. Since the aim of this study was to determine the *Culicoides* abundance in the area and not to compare sites, data for all collection sites were pooled.

4.3.1 Species frequency, abundance and occurrence

In the 261 collections made from February 2007 to May 2010, 76 295 *Culicoides* midges belonging to 20 species were collected (Table 4.3.1).

The number of different *Culicoides* species ranged from a mean of one species per collection during June up to a mean of 10 during September. The most frequent species to be collected was *C. imicola.* It was present in 73.2% of all collections made and represented 50.3% of all the *Culicoides* specimens collected (Table 4.3.1). Based on a combination of abundance in collections and frequency of occurrence this species also had the highest vector rating (61.8%) of all *Culicoides* species collected (Table 4.3.1). Other frequently found species which were present in more than half of the collections included *C. pycnostictus* (60.2%), *C. leucostictus* (59.4%), and *C. bolitinos* (53.3%) (Table 4.3.1). *Culicoides leucostictus* was the second most abundant species and accounted for 23.3% of all the specimens. *Culicoides pycnostictus* and *C. bolitinos* represented 13.8% and 7.1% respectively. *C. imicola* also had the highest vector rating of 61.8%, with *C. pycnostictus* (37.0%) and *C. leucostictus* (41.4%) following close behind. The vector rating of *C. bolitinos* was less than half that of *C. imicola* at 30.2%.

Nine of the 20 *Culicoides* species collected were present in fewer than five of the 261 collections made. These species were collected in low numbers and represented less than 0.1% of all specimens. The vector rating for these species was < 0.1% (Table 4.3.1). *Culicoides* were absent in 42 of the 261 collections made.

Table 4.3.1 Species occurrence, frequency and abundance as determined in 261 light trap collections made near livestock from February 2007 to May 2010 near Bloemfontein in the central Free State.

Culicoides species	Frequency in 261 collections (%)	Total collected (%)	Vector rating (%frequency + %abundance)/ 2
C. imicola	191 (73.2)	38 350 (50.3)	61.8
C. pycnostictus	157 (60.2)	10 501 (13.8)	37.0
C. leucostictus	155 (59.4)	17 751 (23.3)	41.4
C. bolitinos	139 (53.3)	5 437 (7.1)	30.2
C. nivosus	100 (38.3)	2 534 (3.3)	20.8
C. similis	52 (19.9)	521 (0.7)	10.3
C. bedfordi	23 (8.8)	340 (0.4)	4.6
C. exspectator	19 (7.3)	231 (0.3)	3.8
C. neavei	16 (6.1)	180 (0.2)	3.2
C. zuluensis	31 (11.9)	114 (0.1)	6.0
C. magnus	35 (13.4)	305 (0.4)	6.9
C. albopunctatus	3 (1.1)	12 (<0.1)	<0.1
C. milnei	5 (1.9)	9 (<0.1)	<0.1
C. olyslageri	2 (0.8)	3 (<0.1)	<0.1
C. enderleini	2 (0.8)	2 (<0.1)	<0.1
C. brucei	1 (0.4)	1 (<0.1)	<0.1
C. coarctatus	1 (0.4)	1 (<0.1)	<0.1
C. cornutus	1 (0.4)	1 (<0.1)	<0.1
C. nevilli	1 (0.4)	1 (<0.1)	<0.1
C. schultzei	1 (0.4)	1 (<0.1)	<0.1
No *Culicoides* present	42 (16.1)	0	
Total		76 295	

4.3.2 Seasonality in *Culicoides* numbers

The monthly mean numbers of midges collected over the four-year period are shown in Fig. 4.3.1. The monthly mean number of *C. imicola*, the dominant species, per trap night from 2007 to 2010 is summarized in Table 4.3.2.

2007

The surveys started in February 2007 and by November 2007 a total of 93 collections had been made at four of the six collection sites depicted in Fig. 4.2.1. The majority of the collections were made at site 1 (n = 34) and site 4 (n = 33). During 2007 midge

numbers were very low (< 100) (Fig. 4.3.1 A). The maximum number of midges collected in a single light trap was 69 (November). Midges were absent from collections made during June and July (Fig. 4.3.1 A) and from some in April and August (Fig. 4.3.1 A). The maximum number of midges collected during the winter months was two midges in August. This low number and absence of midges was followed by a very slow buildup of numbers after September towards the end of the year (Fig. 4.3.1 A).

Similar to the total number of midges collected *C. imicola* was also only collected in relatively small numbers (Table 4.3.2). These ranged from 0 (April to August) to 38 (March). A mean of 4.7 *C. imicola* were collected per trap during 2007 (Table 4.3.2). The proportion of *C. imicola* compared to the total number ranged from 0.0% (August) to 93.1% (March).

2007/8

From December 2007 up to November 2008 a total of 96 collections were made at two of the six sites (Fig. 4.3.1 B). The majority of the collections were at site 1 (n = 84). During 2008 the average number of midges that were recovered from the light traps was much higher during the first few months of the year than during 2007 (Fig 4.3.1 A & B). Although midges were present in the light traps throughout the winter months the numbers decreased rapidly to a mean of fewer than 20 per collection (June to September). Although midges were absent from collections on some nights during July and August it was still possible to collect up to a 94 on a single night in July. The maximum number of midges collected by a light trap in a single night was 1 695 (March) and 1 632 (April) (Fig. 4.3.1 B).

During 2007/2008 larger numbers of *C. imicola* were collected, ranging from 0 (June to August) to 1 488 (April). A mean of 105.4 *C. imicola* were collected per trap night during 2007/2008 (Table 4.3.2). The proportion of *C. imicola* compared to the total number ranged from 5.0% (July) to 81.9% (April) (Fig. 4.3.2).

2008/9

From December 2008 up to November 2009 a total of 56 collections were made at three of the six sites (Fig. 4.3.1 C). As for the previous two years the majority of

collections were made at site 1 (n = 53). During 2009 the midges built up to a peak of more than a thousand midges in all the collections from March to April (Fig. 4.3.1 C). Despite the relatively large collections made in March and April, midges were absent in five collections made in June and July (Fig. 4.3.1 C). During August the mean number of midges collected was below 100 (Fig. 4.3.1 C). The maximum number of midges during the winter months was 48 (August). The maximum number was 5 880 (April) and from December to May the maximum exceeded 1 000 midges per collection (Fig. 4.3.1 C).

During 2008/2009 the largest number of *C. imicola* was collected, ranging from 0 in June, July and October to 5 560 in April. On average 596.5 *C. imicola* were collected per trap during 2008/2009 (Table 4.3.2). The proportion of *C. imicola* compared to the total *Culicoides* ranged from no midges collected (July) to 94.1% (April) (Fig. 4.3.2).

2009/10

From December 2009 up to the end of the survey in May 2010 a total of 16 collections were made at site 1 (Fig. 4.3.1 D). The survey was terminated in May 2010 when the number of midges dropped to less than 10 (Fig. 4.3.1 D). The minimum number was seven during May and the maximum was 1 000 during February (Fig. 4.3.1 D).

During 2009/2010 the numbers of *C. imicola* collected ranged from six (May) to 810 (February). A mean of 371.8 *C. imicola* were collected per trap during 2009/2010 (Table 4.3.2). The percentage *C. imicola* of the total *Culicoides* ranged from 62.2% (December) to 95.2% (April) (Fig. 4.3.2).

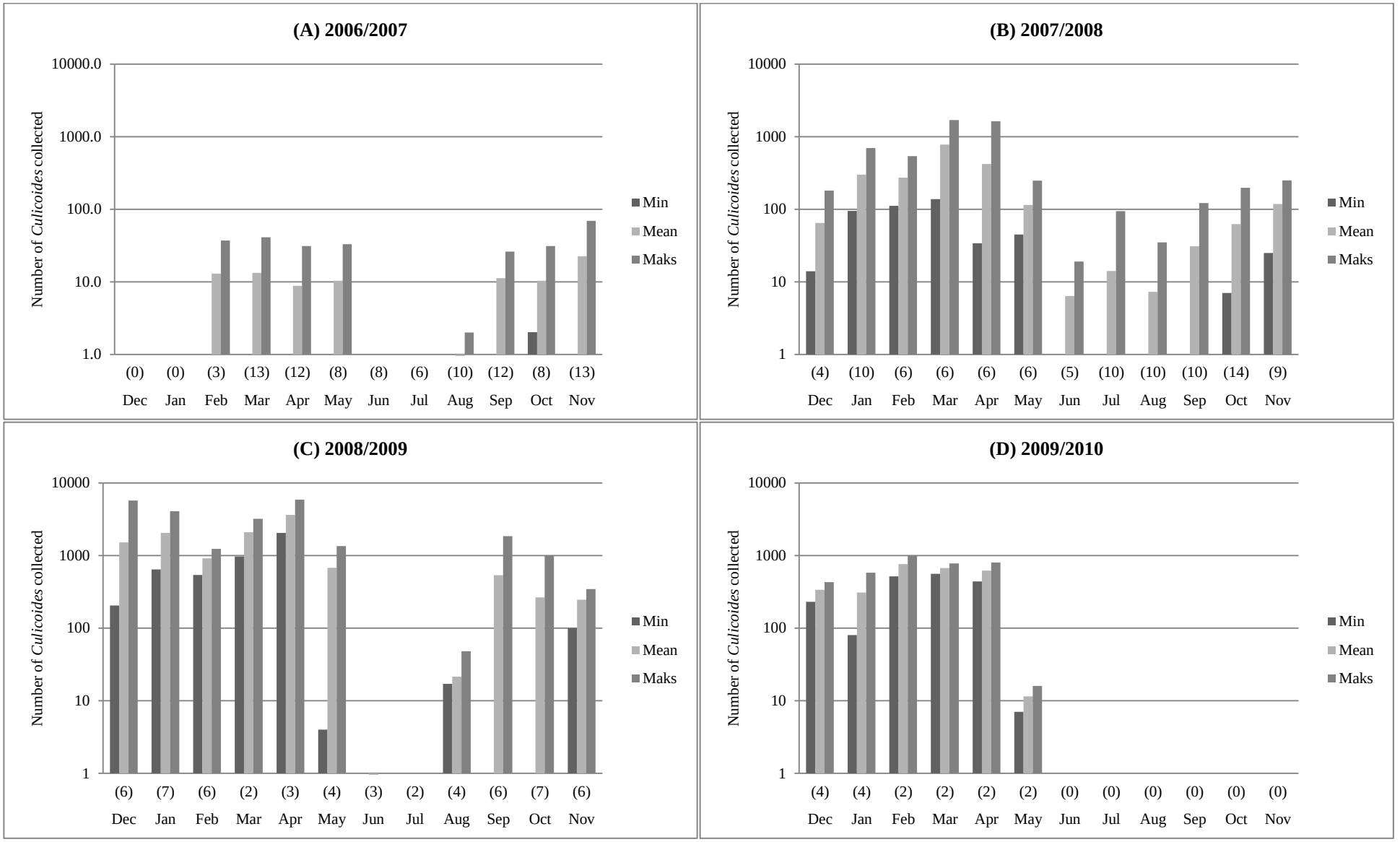

Figure 4.3.1 Mean monthly variation in *Culicoides* numbers, collected by light traps during (A) 2006-2007; (B) 2007-2008; (C) 2008-2009 and (D) 2009-2010 at six locations in the central Free State, as well as the number of collections made during each month.

Table 4.3.2 Monthly mean number of *Culicoides imicola* collected per trap from February 2007 up to May 2010 near Bloemfontein in the central Free State

	Summer			Autumn			Winter			Spring		
Year	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
2006/2007			11.3	12.4	7.8	8.8	0.0	0.0	0.0	2.1	1.5	2.7
(Range)			(1–33)	(1–38)	(0–28)	(0-28)	(0-0)	(0-0)	(0-0)	(1-8)	(2-10)	(1-8)
Number of collections	-	-	3	13	12	8	8	6	10	12	8	13
2007/2008	22.8	107.4	161.7	501.3	344.3	67.7	0.8	0.7	1.2	8.4	12.7	35.7
(Range)	(6-55)	(21-417)	(41-412)	(91-1175)	(8-1488)	(25-143)	(0-3)	(0-6)	(0-4)	(1-53)	(1-45)	(6-110)
Number of collections	4	10	6	6	6	6	5	10	10	10	14	9
2008/2009	309.8	92.9	628.3	1860.0	3 420.0	533.8	0.3	0.0	6.0	57.0	162.0	88.3
(Range)	(44-800)	(30-220)	(160-1 115)	(860-2 860)	(1 820-5 560)	(4-1 110)	(0-1)	(0-0)	(2-13)	(1-180)	(0-750)	(10-240)
Number of collections	6	7	6	2	3	4	3	2	4	6	7	6
2009/2010	210.0	195.0	625.0	602.6	590.3	8.0						
(Range)	(170-310)	(35-360)	(440-810)	(504-702)	(419-762)	(6-10)						
Number of collections	4	4	2	2	2	2	-	-	-	-	-	-
Mean *C. imicola*	**180.9**	**131.8**	**356.6**	**744.1**	**1 090.6**	**154.5**	**0.4**	**0.2**	**2.4**	**22.5**	**58.7**	**42.2**

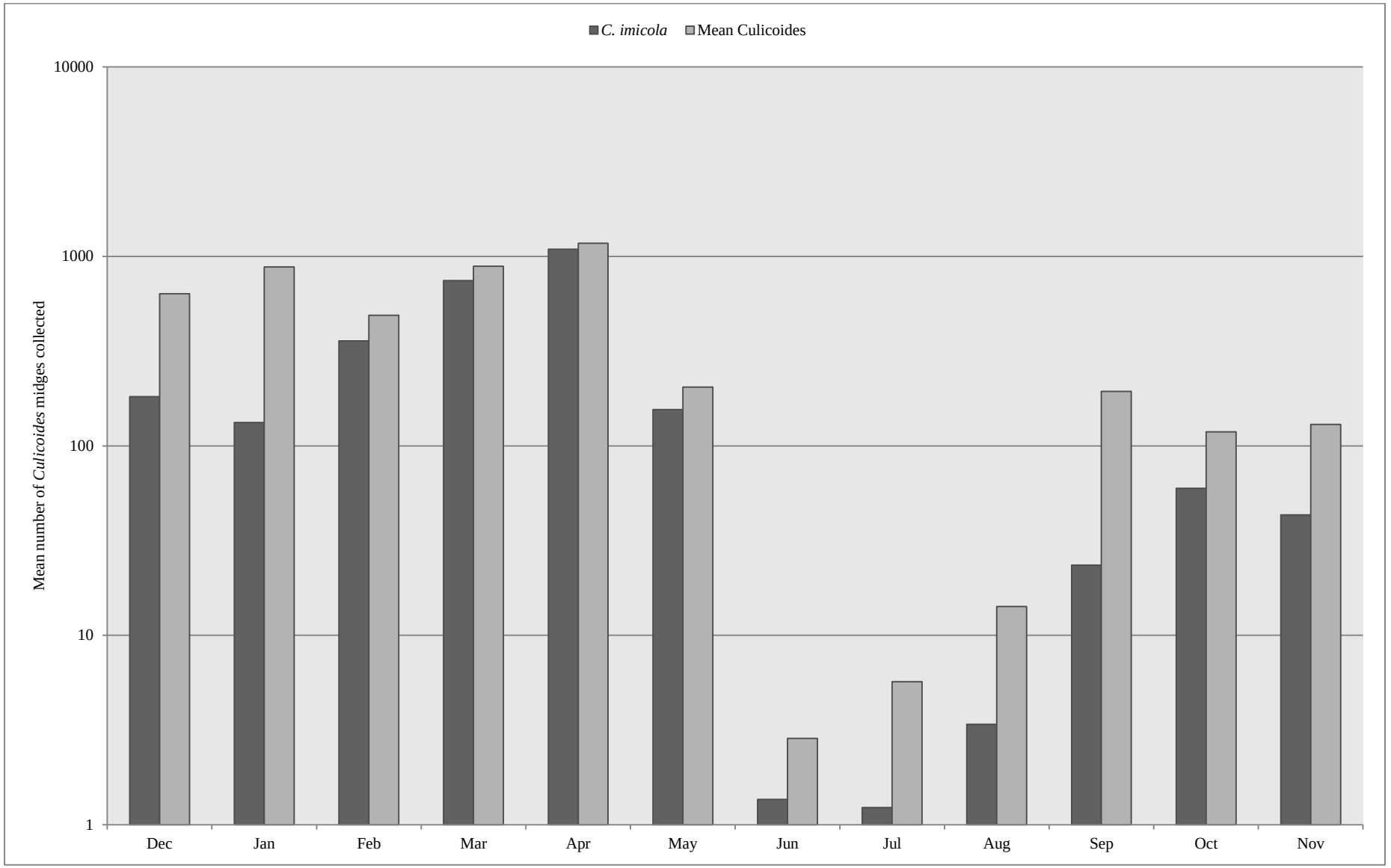

Figure 4.3.2 Mean monthly variation in *Culicoides* and *Culicoides imicola* numbers, collected by FS traps from February 2007 to May 2010 at six locations in the central Free State.

4.3.3 Co-occurrence of *C. imicola* and *C. bolitinos*

In the collections made from 2007 to 2010 (Fig. 4.3.3 A-D), the occurrence of *C. bolitinos* and *C. imicola* followed the same seasonal patterns, but with *C. bolitinos* present in lower numbers. There were times when there were exceptions, during the colder months *C. bolitinos* was sometimes more abundant than *C. imicola*. *Culicoides bolitinos* was the dominant species to be collected in October 2007, June to September 2008 and August 2009.

During 2007 the low numbers of midges collected during the usual peak for the population, influenced the midge survival during the winter and the build-up the following spring (Fig. 4.3.3 A). This can be seen clearly when looking at the collections during the following few years when midges were more abundant.

In 2008 the resilience of *C. bolitinos* and their abundance relative to *C. imicola* in the winter months was clearly visible (Fig. 4.3.3 B). During the coldest months (June to August) *C. bolitinos* was still present in the collections at a mean of approximately ten midges per collection while only single *C. imicola* specimens were found.

During 2009 (Fig. 4.3.3 C) and 2010 (Fig. 4.3.3 D) *C. imicola* was abundant and followed the same pattern as the temperature, the number of *C. imicola* decreasing as the temperatures decreased during the colder months. *Culicoides bolitinos,* however, was absent from the collections during the warmest month (December) of the year.

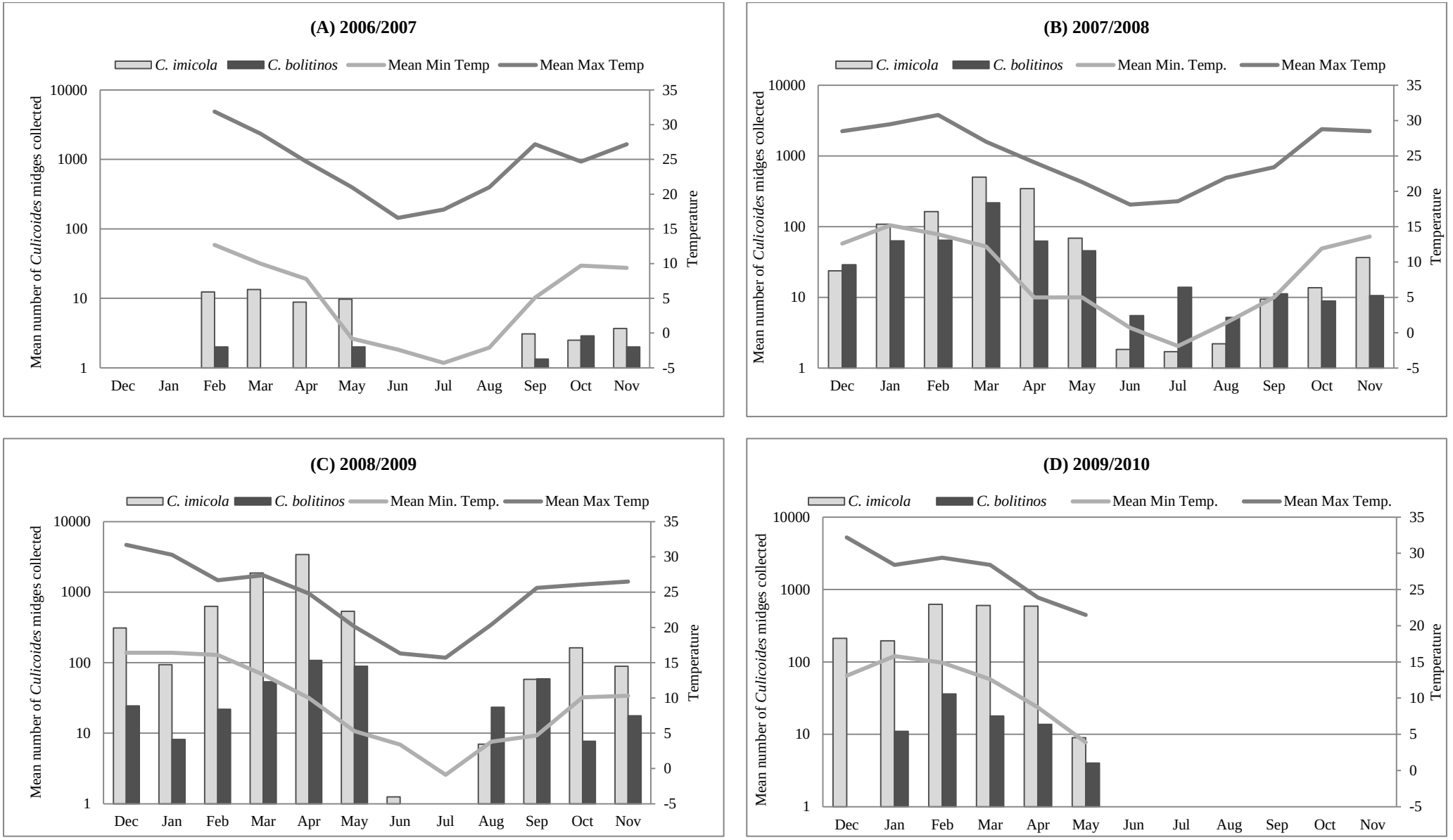

Figure 4.3.3 Mean monthly variation in *Culicoides imicola* and *Culicoides bolitinos* numbers collected, minimum and maximum temperature recorded, during (A) 2006-2007; (B) 2007-2008; (C) 2008-2009 and (D) 2009-2010 at six locations in the central Free State.

4.3.4 Age grading results for *C. imicola*

The number of nulliparous *C. imicola* females collected ranged from zero in the winter months to a maximum of 4 080 during April 2009. The number of parous *C. imicola* ranged from zero in the winter months to a maximum of 1400 during April 2009. The number of parous midges varied considerably between years but the time of the year when the peak in midge numbers was found, namely February to April, remained constant between years (Fig. 4.3.4). During the entire collection period the ratio of parous to nulliparous midges stayed relatively constant, ranging from 0.16 in September to 1.48 in February (Fig. 4.3.4).

The males and freshly blood engorged female *C. imicola* followed the same pattern as the nulliparous and parous midges but at a much reduced level. These groups were never abundant in collections but were only found when large numbers of midges were present. The maximum number of freshly blood engorged *C. imicola* collected was five, during December, February and March. The maximum number of gravid *C. imicola* females was 65 (March 2008). The maximum number of *C. imicola* males collected was 120 (March 2009).

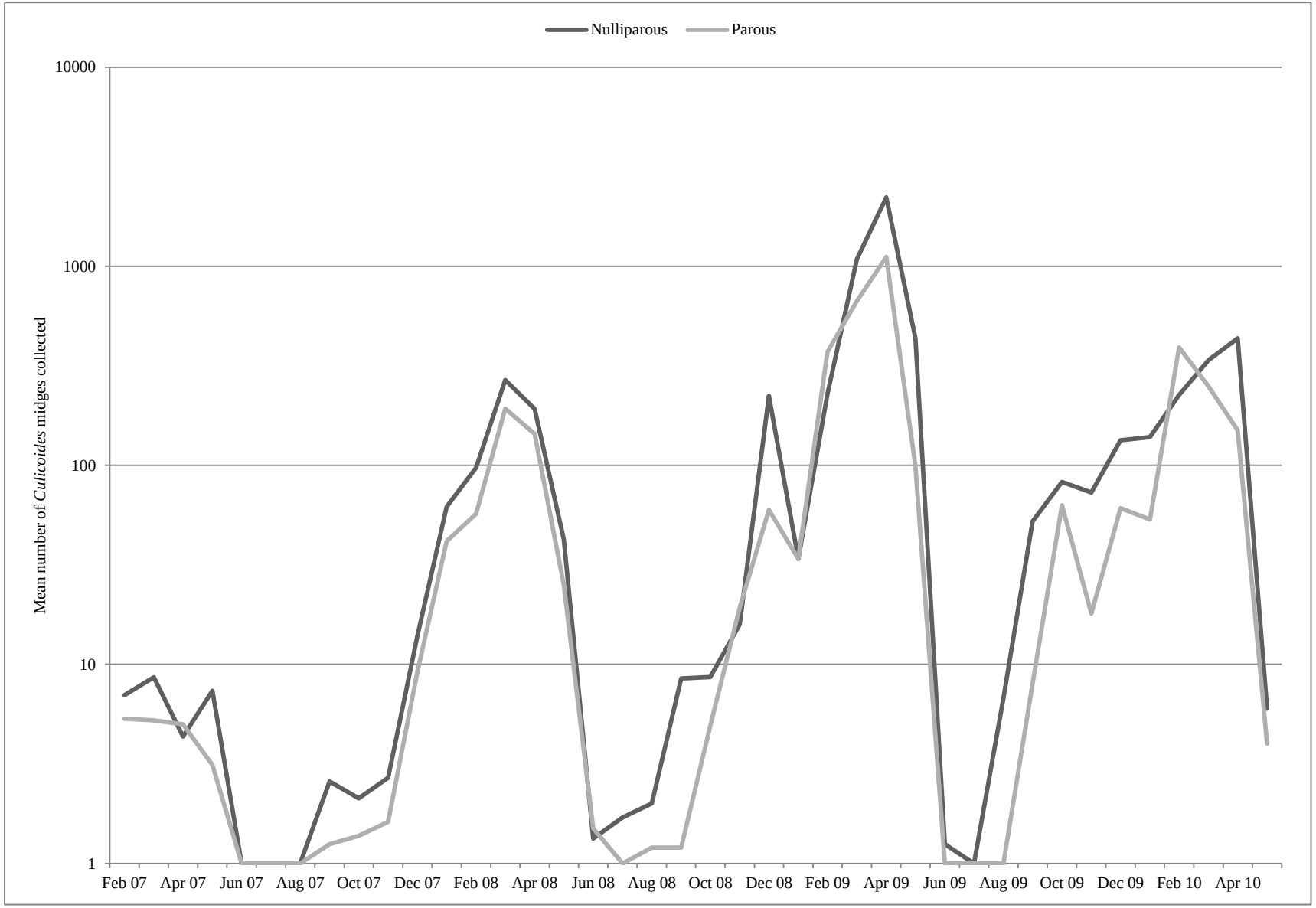

Figure 4.3.4 Mean monthly variation in parous and nulliparous *Culicoides imicola* numbers, collected from February 2007 to May 2010 at six locations in the central Free State.

4.3.5 Effects of rainfall on *Culicoides* numbers

The rainfall recorded during the collection period varied greatly (Fig. 4.3.5). The total rainfall of 482 mm recorded from September 2007 to May 2008 was lower than the 30 year average of 524 mm. From September 2008 to May 2009 a total of 559 mm and from September 2009 to May 2010 a total of 641 mm was recorded, both totals higher than the 30-year mean.

On average the highest rainfall was recorded during November (118.7 mm) and the lowest was recorded in August (3.7 mm). During 2009 and 2010 the highest monthly rainfall was recorded during January and February (Fig. 4.3.5).

During the early months (February to April) of 2007 the numbers of *Culicoides* midges were relatively low, fewer than 15 midges per trap (Fig. 4.3.5 A). In this period the monthly rainfall reached a peak of only 45.3 mm.

In the beginning of 2008 the rainfall was constant at a monthly average of just below 50 mm and increased to over a 100 mm during March (Fig. 4.3.5 B). This increase in rainfall coincides with the peak number of midges for 2008 when the average was more than 500 per trap. During the winter months the rainfall decreased and the midge numbers also decreased (Fig. 4.3.5 B). After winter the rainfall did not increase until November and the midge numbers also increased slowly (Fig. 4.3.5 B).

In the beginning of 2009 the average monthly rainfall was higher than 125 mm per month (Fig. 4.3.5 C) and the *Culicoides* numbers increased to an average of more than 1 800 in March and 3 400 in April. During April and May, the numbers remained high, even though the rainfall had decreased rapidly (Fig. 4.3.5 C). During the winter rainfall dropped to less than 22 mm per month and the midges also disappeared from the collections. Towards the end of 2009 the midge numbers increased rapidly as the monthly rainfall also increased (Fig. 4.3.5 C).

During 2010 (Fig. 4.3.5 D) midge numbers continued to increase while the rainfall remained higher than 100 mm per month. During April the rainfall decreased to below 10 mm and the numbers decreased to such an extent that the collections made at the end of May were midge-free.

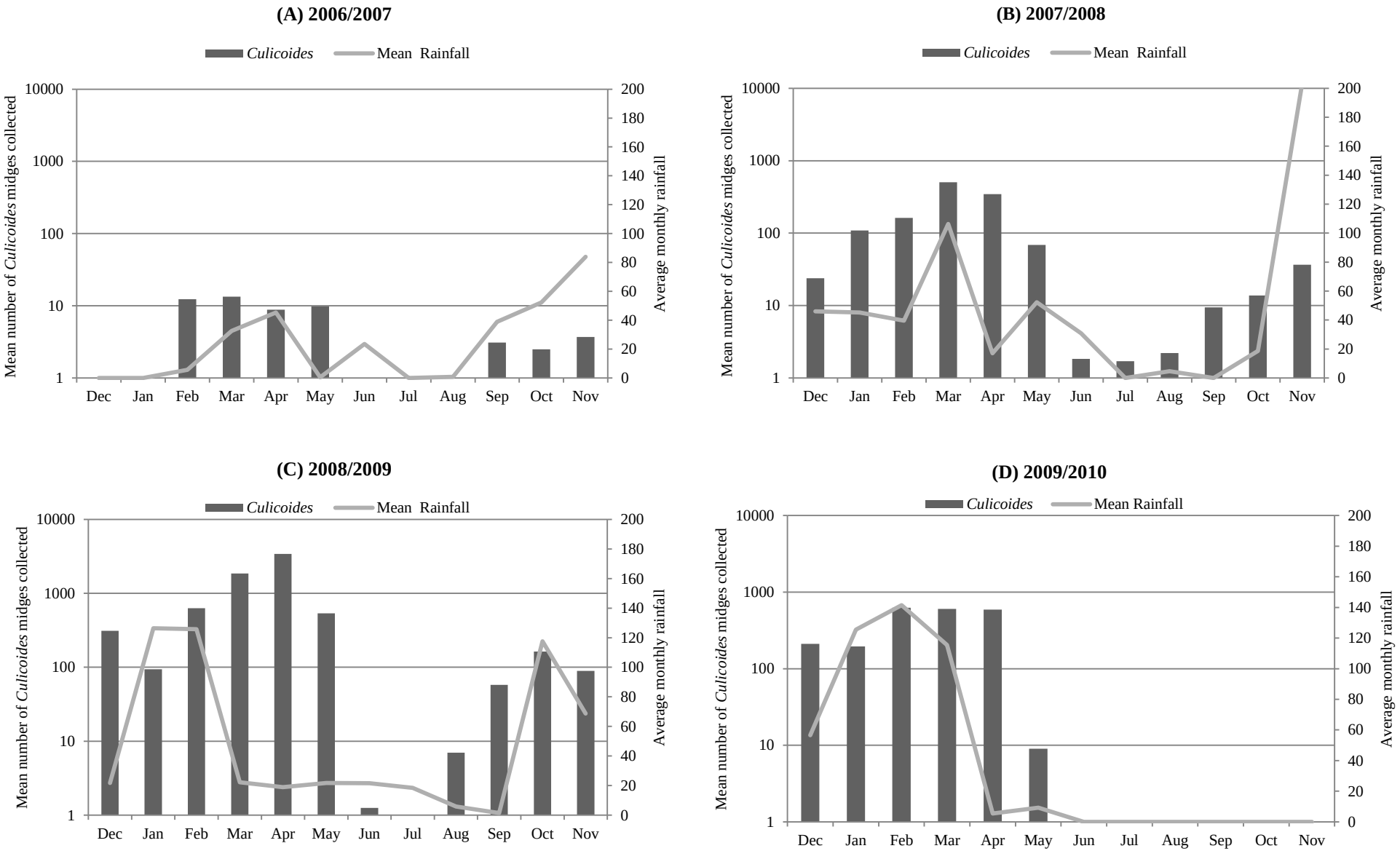

Figure 4.3.5 Mean monthly variation in *Culicoides* numbers collected and monthly rainfall recorded, during (A) 2006-2007; (B) 2007-2008; (C) 2008-2009 and (D) 2009-2010 at six locations in the central Free State.

4.3.6 Effects of temperature on *Culicoides* numbers

The mean minimum monthly temperature recorded ranged from -2.4°C in July to 15.8°C in January (Fig. 4.3.6). During 2007 the minimum temperatures for the winter months (June to August) averaged at -2.9°C as opposed to an average of 0.1°C during 2008 and 2.1°C during 2009. The average maximum temperatures ranged from 17.0°C (June) to 30.8°C (December). The monthly maximum temperatures for each year were relatively constant over the four-year sampling period (Fig 4.3.6).

In 2007 the collections made during June to August were *Culicoides*-free, the minimum temperatures for these three months were below zero (Fig. 4.3.6 A). In 2008 (Fig. 4.3.6 B) the winter temperatures were more moderate and the midges did not disappear from the collections but were present throughout the winter, although in low numbers.

The maximum temperatures from February 2009 up to May 2009 was more moderate and co-incided with the buildup of large populations of *Culicoides* (Fig. 4.3.6 C). Although the minimum winter temperatures were relatively moderate, the maximums were lower than in previous years and the midges also disappeared from the collections.

In the beginning of 2010 the maximum temperatures were on average 2°C higher than those of 2009 (Fig. 4.3.6 D). This could be an explanation for the lower numbers of midges during the same period of 2009.

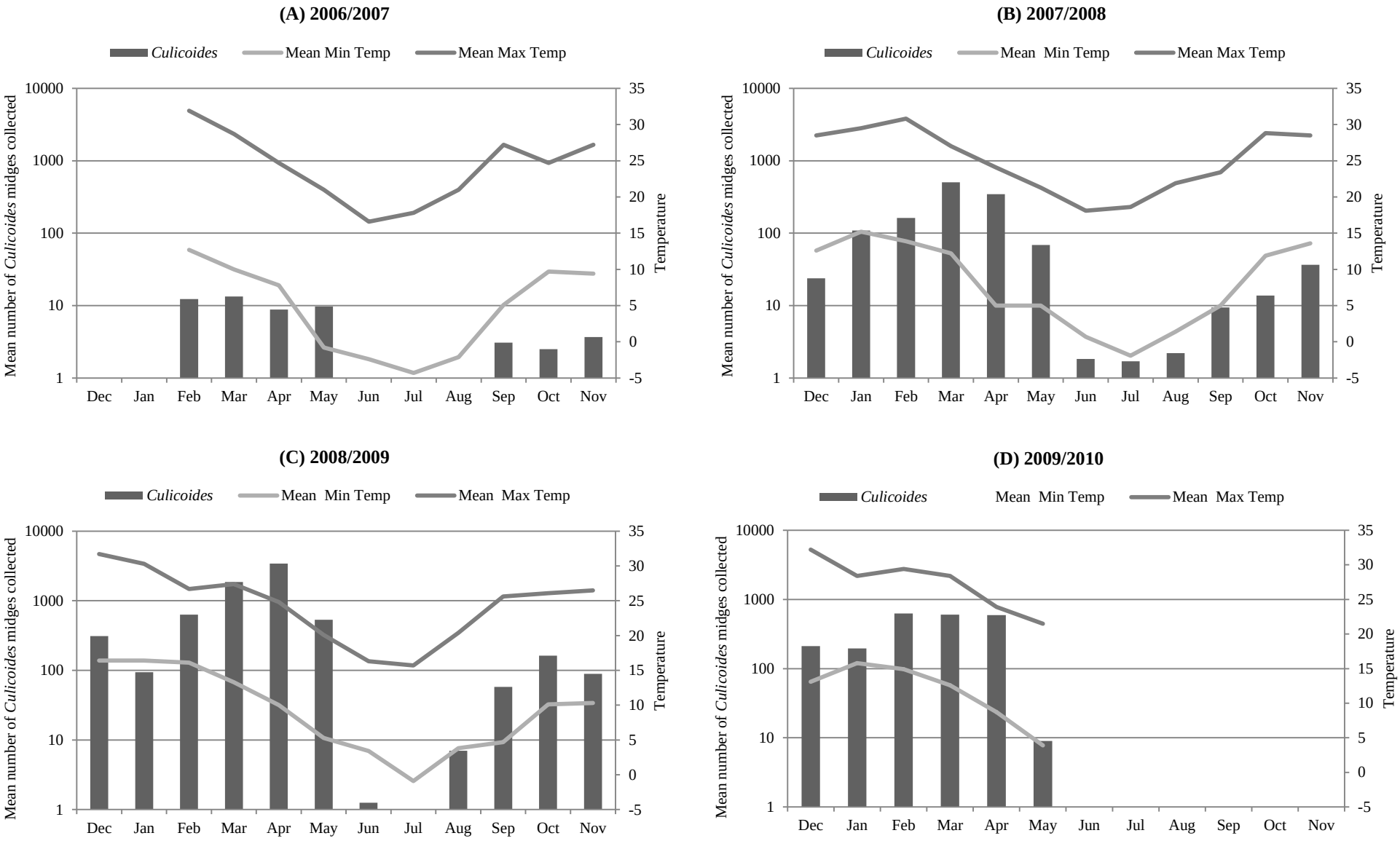

Figure 4.3.6 Mean monthly variation in *Culicoides* numbers collected, minimum and maximum temperature recorded, during (A) 2006-2007; (B) 2007-2008; (C) 2008-2009 and (D) 2009-2010 at six locations in the central Free State.

4.4 Discussion

The central Free Sate is relatively high lying and can be classified as a semi-dry summer rainfall area with characteristically cold dry winters. The average winter temperatures in the area were much lower than those of the traditional AHS endemic areas in the north of the country. Summer temperatures are, however, comparable to the rest of South Africa. Despite the relatively harsh conditions more than 100 cases of AHS were reported between 2005/6 and 2010/11 in the Free State Province (AHS Trust, 2012). Contrary to the general view that outbreaks occur only every five years outbreaks were reported every year in the province (AHS Trust, 2012).

4.4.1 Species frequency, abundance and occurrence

A total of 20 *Culicoides* species were found in light trap collections made from February 2007 up to May 2010 in the area. In this survey light trap collections were exclusively done near livestock and *C. imicola,* a proven vector of AHSV and BTV (Mellor *et al*., 2000) was the most abundant *Culicoides* species to be collected. This was also the most frequently found species near livestock in the central Free State, indicating the level of significance of this species for disease transmission.

The only previous study to be done in the central Free State was that of Venter *et al*. (1996) conducted at the Glen Agricultural College near Bloemfontein. Only eight collections were made during the summer months of 1983/84 (Venter *et al.,* 1996). Midges belonging to 14 species were found and the mean collection size was 1 183 *Culicoides* midges. The maximum number collected was 6 740 midges and *C. pycnostictus* was dominant. Since these collections were made using the more effective OP 220V trap (Chapter 2) and only in summer it is not possible to compare that data with the present information.

The number of midges collected and the *Culicoides* species composition as determined using light traps are only an indication of the numbers and species of midges present in the area and not a direct indication of the midge's biting rate (Carpenter *et al.,* 2008; Gerry *et al*., 2009). Other frequently found species that also regularly feed on livestock were *C. pycnostictus* and *C. bolitinos*. The bird-feeding species *C. leucostictus* was also often present in collections.

Based on abundance in light trap collections and their frequency, the species with the highest vector rating in the present survey were *C. imicola*, *C. pycnostictus* and *C. leucostictus*.

Culicoides imicola has been shown to be the most abundant livestock-associated species in South Africa (Venter *et al.*, 1996; Venter & Meiswinkel, 1994; Meiswinkel *et al*., 2004). It is widely distributed and is more abundant in warm, low-lying areas than in areas characterized by cold winters and severe frost (Venter *et al*., 1996). *Culicoides imicola* is also less abundant in dry, warm areas *e.g*. Upington (Venter *et al*., 1996) and in the sandy dune fields adjacent to Port Elizabeth in the Eastern Cape Province (Meiswinkel, 1997). According to precipitin tests, *C. imicola* feeds predominantly on cattle and sheep (Meiswinkel *et al*., 2004), and is still the only proven vector of BT and AHS in South Africa (Du Toit, 1944; Venter *et al*., 1996). Because of its wide distribution, strong association with livestock and high abundance, *C. imicola* is rated as the most important vector of AHSV and BTV in South Africa (Venter *et al*., 1996).

The second most abundant species to be collected was *C. leucostictus*. This species has a wide distribution and is regularly found near livestock (Venter *et al.*, 1996). It can become abundant in collections made near poultry in the absence of livestock (Venter *et al.*, 1996; Labuschagne *et al*., 2007). This species feeds predominantly on birds and poultry and can therefore be a vector of bird-related pathogens (Nevill & Anderson, 1972; Nevill *et al.*, 1988; Meiswinkel *et al*., 2004). Although laboratory studies show that it may be susceptible to infection with orbiviruses (Venter *et al*., 2011) its role as a vector of livestock viruses is not clear.

The third most abundant species to be collected, *C. pycnostictus*, also feeds mainly on poultry, but may feed on cattle and horses (Jupp *et al*., 1980; Braverman & Phelps, 1981; Meiswinkel *et al.*, 2004) and is one of the most abundant species in the Free State (Venter *et al*., 1996). It can be considered as a potential vector of BTV, because BTV serotypes 6 and 24 have been isolated from field-collected *C. pycnostictus* (Nevill *et al.*, 1992). Similar to *C. leucostictus* laboratory studies show that it may be susceptible to infection with orbiviruses (Venter *et al*., 2011) but its role as a vector of livestock viruses is also not clear.

4.4.2 Seasonality of *Culicoides imicola* in the Bloemfontein area

On average *C. imicola* represents 50.3% of midges collected in the present survey. Its representation varies from 84.1% and 90.3% in March and April to 1.7% and 14.9% in July and August. From the data presented in Fig 4.3.2 it is, however, clear that the total numbers of *Culicoides* collected fluctuated as the numbers of *C. imicola* captured fluctuated.

A distinct seasonal pattern was observed when assessing the numbers of *Culicoides* collected. Midges virtually disappeared from light trap collections during the colder unfavorable winter months then building up to a population peak during the late summer and autumn.

In the frost-free areas of South Africa *Culicoides* midges occur throughout the year in large numbers. In the central Free State midge numbers declined considerably during the winter months (June to August) and in some years they even disappeared completely during June and July of 2007 and July of 2009. This is due to their reduced activity and slower development during the colder months (Meiswinkel *et al*., 2004).

As mentioned, light traps are not the most effective tool for monitoring the midge's biting rate, but the peak of *C. imicola* numbers strongly correlates with periods of high risk for disease transmission in the central Free State. Therefore light traps are still one of the most efficient and practical methods for monitoring *Culicoides* occurrence and the assessment of midge activity to determine possible periods of high risk for disease transmission. It is, however, clear that light trap results need to be compared to what is known about the biology (*e.g.* host preference) of the *Culicoides* species collected to determine the risk of diseases spreading and occurring in an area.

4.4.3 Co-occurrence of *C. imicola* and *C. bolitinos*

The fourth most dominant species in the present survey, *C. bolitinos*, is like *C. imicola* also a member of the subgenus *Avaritia*. *Culicoides bolitinos* is usually the most abundant *Culicoides* species to be collected in the colder high-lying areas of the country, *e.g.* the eastern Free State (Venter *et al*., 1996; Meiswinkel & Paweska, 2003). This species is strongly associated with bovines, breeding in the dung of large

herbivores (Meiswinkel, 1989; Venter *et al*., 1996) and was shown to feed on cattle, sheep and horses (Nevill *et al*., 1988). During 1998, approximately 100 horses died of AHSV in the cooler parts of the central region of South Africa (Meiswinkel & Paweska, 2003). More than 150 000 *Culicoides* of 27 species were captured but *C. imicola* constituted <1% of the total *Culicoides* captured when compared to the 65% *C. bolitinos* (Meiswinkel & Paweska, 2003). Isolations of AHSV have been made from *C. bolitinos* (Meiswinkel & Paweska, 2003).

To date, however, *C. imicola* remains the only confirmed vector species of both AHSV and BTV in southern Africa. *Culicoides bolitinos* is suspected of being a vector of both these diseases as this species also feeds on livestock and isolations of both viruses from this midge have been made. Also laboratory infections of *C. bolitinos* with the viruses (Mellor *et al*., 2000) and a relatively similar distribution to that of *C. imicola* in southern Africa (Meiswinkel, 1989) make it a strong candidate. *Culicoides bolitinos* also occurs in colder areas where *C. imicola* is less abundant and it has even been collected in areas with disease occurrence where *C. imicola* was absent (Meiswinkel & Paweska, 2003). This indicated that *C. bolitinos* might act as the primary vector for disease transmission in colder areas.

The population of *C. imicola* grows exponentially during the warmer months and disappears during the colder winter months. The population of *C. bolitinos* remained relatively constant at modest numbers and even during the coldest months of the year they did not disappear from the collections. This was again an indication and confirmation of the importance of *C. bolitinos* during cold times and in colder areas as a possible over wintering vector to maintain pathogens in these specific areas in the absence of *C. imicola*. The occurrence of *C. bolitinos* during the cold winter months is most probably a result of its breeding in cattle dung which is a constant source of breeding habitat and not as much affected by the environmental temperature and rainfall.

4.4.4 Age grading

Since transovarial transmission of orbiviruses is considered not to be the norm in the genus *Culicoides,* orbiviruses can only be transmitted by infected females after the completion of the gonotrophic cycle (Akey & Barnard, 1983; Venter *et al*., 1997). The

ratio of parous (midges that have completed a gonotrophic cycle) to nulliparous (midges that have not yet completed a cycle) can therefore be used as an indication of the vector potential of a *Culicoides* population.

As summer progresses the ratio of parous *Culicoides* females increases which signifies a higher vector potential towards the end of the summer (Venter *et al*., 1997). This coincides with the time of occurrence of BT and AHS outbreaks in most of the temperate zones of South Africa (Venter *et al*., 1997). The low numbers of *Culicoides* in the winter months is mostly due to reduced adult activity and slower development of the immature stages as a result of low temperatures (Venter *et al*., 1997). This may also be due to the lower rainfall and subsequent less larval and immature habitats during the drier winter (Venter *et al*., 1997).

Throughout the year whenever *Culicoides* midges were present in collections, nulliparous and parous midges were also present. This is an indication that midges continually breed throughout the favorable period of each year (Walker, 1977). This is also an indication of overlapping generations. The presence of males of *C. imicola* year round indicates that midges can breed year round, and the bloodfed midges also suggest feeding activity throughout the year. The low numbers of males and bloodfed females in the collections is due to the drawbacks of light traps as discussed in Chapter 2.

Disease transmission by *C. imicola* is, to some extent, limited to the periods when the midge population peaks because of their low infection rate. In the central Free State there is only a certain period of time (February to May) when the midge numbers are high enough to create a period of high risk for disease transmission. When factoring in the influence of the cold winters, dry conditions and the infection rate of *C. imicola* for AHSV the cases and outbreaks of the disease coincide with years of above average rainfall. Therefore, the role of a reservoir host to cycle and maintain the disease during unfavorable times is an important factor to keep this disease in the central Free State. The exception to this general observation is in areas with year round breeding sites like dams and rivers, etc.

4.4.5 Effects of rainfall on *Culicoides* numbers

Midge dependence on rainfall can be seen when comparing the rainfall patterns in the central Free State to the number of midges collected between 2007 and 2010. A distinct peak was observed during the rainy season and numbers decreased in the winter months when the rainfall decreased to months with no rainfall at all.

Culicoides midges, and especially the larval stages, are extremely dependent on moisture to exist and flourish. They need wet marshy areas as breeding sites and also to reach high numbers. Rainfall combined with favorable temperatures had the most noticeable impact on the number of midges that occurred in an area. The increased relative humidity and availability of breeding sites during the rainy season led to a rapid increase in the numbers of *Culicoides* midges collected in the light traps. This was also the key time in the disease cycle of midges, the peak time for disease transmission and a resulting higher vector potential because of the larger number of active adult midges. The midge numbers decreased during the winter months when the monthly rainfall decreased and suitable breeding sites became scarce. *Culicoides imicola* have been found to breed in wet, organically enriched kikuyu (*Pennisetum clandestinum*) pastures. These suitable habitats expand during years of heavy rains (Meiswinkel *et al*., 2004). High rainfall can also influence the numbers of *C. imicola* negatively and lead to pupae drowning and delay the emergence of the next generation (Nevill, 1967). This will lead to an increase in the number of parous females, but the higher rainfall will lead to more breeding sites and this will again increase the number of nulliparous midges (Venter *et al*., 1997).

4.4.6 Effects of temperature on *Culicoides* numbers

Environmental temperature will influence the development rate and thereby the number of generations produced and the adult population size (Mullens *et al*., 2004). The rearing temperature influences adult size and the ambient temperature also influences adult survivorship as temperature governs the gonotrophic cycle length and thus adult feeding frequency and viral transmission potential (Mullens *et al*., 2004). In the adult midge temperature also regulates viral infection and replication (Paweska *et al*., 2002; Mullens *et al*., 2004).

The nocturnal activity of midges also limits their resistance to the colder times as the night temperatures are on average approximately 15°C lower than day temperatures. During the winter months when the minimum temperatures fell to below 0°C the midge population was at its lowest and even absent from collections. This absence of midges from light trap collections may be as a result of their inability to fly during the cold nights, not necessarily due to their absence. The midge population peaks during the late summer when temperatures are moderate with minimums ranging between 10°C and 15°C and maximum temperatures ranging between 20°C and 30°C. Although the winter temperatures at night time can drop to below freezing point, the temperature during the day can still reach an average of 15°C. It is therefore possible that midges can become active in late afternoons or early in the evening before the light traps are fully effective as was observed for some British species (Nathan, 1981).

The proven orbivirus vector *C. imicola* was abundant in the central Free State, and occurred year round, even during the colder months in low numbers. This was an important discovery when looking at disease transmission and the potential for disease-free periods. To ensure that the transmission of AHSV is successfully broken, vector-free periods must be of longer duration than the maximum period of viraemia in the local susceptible vertebrate population (Mellor, 1994). However, the occurrence of *C. imicola* even in low numbers during winter months is an indication of a possible threat for disease transmission throughout the year. Nineteen other species of *Culicoides* were also collected in the central Free State, some of which may be additional vector species of BT and AHS. No midge-free periods were identified, but there were periods of lower activity during which animals had a lower possibility of being infected. Viruses can overwinter in the surviving adult midges which then could spread them when favorable conditions return. Horse owners and sheep farmers should therefore be aware of the risk for disease transmission and protect their animals accordingly.

4.5 References

African Horse Sickness Trust. 2012. African horse sickness. Online at: http://www.africanhorsesickness.co.za (accessed 2 January 2012).

Akey, D.H. & Barnard, D.R. 1983. Parity in airborne populations of the biting gnat *Culicoides variipennis* (Diptera: Ceratopogonidae) in Northeastern Colorado. *Environmental Entomology*, **12**, 91-95.

Baylis, M., Mellor, P.S. & Meiswinkel, R. 1999. Horse sickness and ENSO in South Africa. *Nature*, **397**, 574.

Bosman, P., Bruckner, G.K. & Faul, A. 1995. African horse sickness surveillance systems and regionalization/zoning: the case of South Africa. *Revue scientifique et technique, Office International des Epizooties,* **14**, 645-653.

Braverman, Y. & Phelps, R.J. 1981. Species composition and blood-meal identification in samples of *Culicoides* (Diptera : Ceratopogonidae) collected near Salisbury, Zimbabwe in 1976-77. *Journal of the Entomological Society of South Africa*, **44**, 315-323.

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. & Mellor, P. 2008. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector? *Journal of Applied Ecology*, **45**, 1237-1245.

Du Toit, R.M. 1944. The transmission of Blue-Tongue and Horse Sickness by *Culicoides. Onderstepoort Journal of Veterinary Science and Animal Industry*, **19**, 7-16.

Coetzer, J.A.W. & Guthrie, A.J. 2004. African horse sickness, in: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, pp. 1231–1246.

Gerry, A.C., Sarto I Monteys, V., Moreno Vidal, J.O., Francino, O. & Mullens, B.A., 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in northeastern Spain in relation to midge capture using UV light and carbon dioxide-baited traps. *Journal of Medical Entomology*, **46**, 615-624.

Guthrie, A.J. 1999. Regionalisation of South Africa for African horse sickness. In *Proceedings of the 8th International Conference on Equine Infectious Diseases* (ed.

by U. Wernery, J.F. Wade, J.A. Mumford and O.-R.Kaaden), pp. 376-379. 23-26 March 1998, United Arab Emirates, Dubai.

Jupp, P.G., McIntosh, B.M. & Nevill, E.M. 1980. A survey of the mosquito and *Culicoides* faunas at two localities in the Karoo region of South Africa with some observations on bionomics. *Onderstepoort Journal of Veterinary Research*, **47**, 1-6.

Labuschagne, K., Gerber, L.J., Espie, I. & Carpenter, S. 2007. *Culicoides* biting midges at the National Zoological Gardens of South Africa. *Onderstepoort Journal of Veterinary Research,* **74**, 343-347.

Meiswinkel, R. 1989. Afrotropical *Culicoides*: a redescription of *C.* (*Avaritia*) *imicola* Kiefer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C.* (*A.*) *bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research,* **56**, 23-39.

Meiswinkel, R. 1997. Discovery of a *Culicoides imicola*-free zone in South Africa: preliminary notes and potential significance. *Onderstepoort Journal of Veterinary Research,* **64**, 81-86.

Meiswinkel, R. & Paweska, J.T. 2003. Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. *Preventive Veterinary Medicine*, **60**, 243-253.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: *Infectious Diseases of Livestock* (ed. by J.A.W. Coetzer and R.C. Tustin), pp. 93-136. Oxford University Press, Cape Town.

Mellor, P.S. 1994. Epizootiology and vectors of African horse sickness virus. *Comparative Immunological and Microbiological Infectious Disease*, **17**, 287-296.

Mellor, P.S., Boorman, J. & Baylis, M. 2000. *Culicoides* biting midges: their role as arbovirus vectors. *Annual Review of Entomology*, **45**, 307-340.

Mullens, B.A., Gerry, A.C., Lysyk, T.J. & Schmidtmann, E.T. 2004. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context. *Veterinaria Italiana*, **40**, 160-166.

Nathan, M.B. 1981. A study of the diurnal biting and flight activity of *Culicoides phlebotomus* (Williston) (Diptera: Ceratopogonidae) using three trapping methods. *Bulletin of Entomological Research*, **71**, 121–128.

Nevill, E.M. 1967. Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. M.Sc. (Agric.) thesis, University of Pretoria, Pretoria.

Nevill, E.M. & Anderson, D. 1972. Host preference of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin test and light trap catches. *Onderstepoort Journal of Veterinary Research*, **39**, 147-152.

Nevill, E.M., Venter, G.J. & Edwardes, M. 1992. Potential *Culicoides* vectors of livestock orbivirus. In *Bluetongue, African horse sickness and related orbiviruses Proceedings of the 2nd International Symposium* (ed. by T.E.Walton and B.I. Osburn), pp. 306-313. CRC Press, Boca Raton, Florida.

Nevill, E.M., Venter, G.J., Edwardes, M., Pajor, I.T.P., Meiswinkel, R. & Van Gas, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **55**, 101-106.

Paweska, J.T., Venter, G.J. & Mellor, P.S. 2002. Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Medical and Veterinary Entomology*, **16**, 10–21.

Venter, G.J. & Meiswinkel, R. 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort Journal of Veterinary Research*, **61**, 327-340.

Venter, G.J., Nevill, E.M. & Van der Linde, T.C. 1996. Geographical distribution and relative abundance of stock associated *Culicoides* species (Diptera: Ceratopogonidae) in southern Africa, in relation to their potential as viral vectors.*Onderstepoort Journal of Veterinary Research*, **63**, 25-38.

Venter, G.J., Nevill, E.M. & Van der Linde, T.C. 1997. Seasonal abundance and parity of stock-associated *Culicodies* species (Diptera: Ceratopogonidae) in different climatic regions in southern Africa in relation to their viral vector potential. *Onderstepoort Journal of Veterinary Research*, **64**, 259-271.

Venter, G.J. & Sweatman, G.K. 1989. Seasonal abundance and parity of *Culicoides* biting midges associated with livestock at Roma, Lesotho (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **56**, 173-177.

Venter, G.J., Wright, I., Del Rio, R., Lucientes, J. & Miranda, M.A. 2011. The susceptibility of *Culicoides imicola* and other South African livestock-associated *Culicoides* species to infection with bluetongue virus serotype 8. *Medical and Veterinary Entomology*, **25**, 320-326.

Walker, A.R. 1977. Adult lifespan and reproductive status of *Culicoides* species (Diptera: Ceratopogonidae) in Kenya, with reference to virus transmission. *Bulletin of Entomological Research*, **67**, 205-215.

Wittmann, E.J., Mellor, P.S. & Baylis, M. 2001. Using climate data to map the potential distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Revue scientifique et technique, Office International des Epizooties*, **20**, 731-740.

SOUTH AFRICAN WEATHER SERVICE

5. THE DIFERENTIAL ATTRACTION OF *CULICOIDES* SPECIES TO LIVESTOCK HOSTS

5.1 Introduction

The biting rate of a specific *Culicoides* species is one of the critical factors determining the vector capacity of a species (Mullens *et al.,* 2004). Host preferences of midges will therefore be an important factor when studying their vector capacity. For example, *Culicoides* species with a specific host preference for equines will be more effective in transmitting AHSV than species that prefer to feed on birds. A species can be very abundant and highly susceptible to a virus but if they do not feed on a susceptible host they will be of lesser importance.

In South Africa, more than 120 species of *Culicoides*, of which approximately 20 are regularly collected near livestock have been identified (Nevill *et al.,* 1992b). Of these 20 *C. imicola* is by far the most abundant species to be collected near cattle, horses, sheep and pigs (Nevill *et al.,* 1992a), usually accounting for more than 90% of all *Culicoides* species collected near livestock (Venter *et al.,* 1996; Meiswinkel *et al*., 2004). The relatively strong host preference of *C. imicola* for large mammals is further illustrated by a substantial drop in numbers if light trap collections are made near poultry or other birds in the absence of bigger mammals (Nevill & Anderson, 1972; Venter *et al*., 1996; Labuschagne *et al.,* 2007). Cumulative blood meal analyses confirmed that this species feed on cattle, sheep and horses (Meiswinkel *et al*., 2004). Of 1 021 blood-engorged *C. imicola* females tested only nine were found to have fed on avian hosts (Meiswinkel *et al.,* 2004).

Other *Culucoides* species which are regularly collected with light traps near livestock in South Africa include *C. zuluensis, C. magnus, C. bolitinos, C. leucostictus, C. pycnostictus, C. nivosus, C. milnei, C. neavei, C. enderleini, C. subschultzei, C. onderstepoortensis* and *C. gulbenkiani* (Nevill *et al.,* 1988; Venter *et al.,* 1996; Meiswinkel *et al.,* 2004). Cumulative blood meal analyses of engorged females showed that all of these species, except *C. leucostictus,* will feed on cattle, sheep and horses (Meiswinkel *et al*., 2004). It was furthermore shown that *C. leucostictus,* together with *C. pycnostictus,* can be collected in bigger numbers at poultry and other birds than at large mammals (Nevill & Anderson, 1972; Labuschagne *et al.,* 2007).

The host preference of these two species for birds is also reflected in blood meal analyses of engorged females. Sixteen of 22 *C. pycnostictus* and all of 61 *C. leucstictus* tested were shown to have fed on an avian host (Meiswinkel *et al.,* 2004). The host preference of these species will, however, not exclude them as potential vectors of AHSV and BTV. It was shown that a small percentage of *C. pycnostictus* will feed on horses, cattle and pigs (Meiswinkel *et al.,* 2004). BTV was furthermore isolated from field-collected *C. pycnostictus* (Nevill *et al.,* 1992a) and artificial infection studies show *C. leucostictus* to be susceptible to infection with AHSV (Venter *et al.,* 2009).

Specific host preferences in the genus *Culicoides* were highlighted by studies in the Kruger National Park, South Africa, that showed five species of *Culicoides* of the subgenus *Avaritia* that feed on elephants also breed in elephant dung (Meiswinkel & Braack, 1994). *Culicoides bolitinos,* a species that breeds in bovine dung, has been found to disperse together with slow moving herds of buffalo in the Kruger National Park (Meiswinkel *et al.,* 2004).

The aim of the present study was to determine which of the abundant *Culicoides* species in South Africa will frequent different livestock species in especially the central Free State.

5.2 Material and Methods

5.2.1 Preference of midges for different livestock

Midges were collected using 8W down draught 12V and 220V FS light traps as described in Chapter 2. Collections of more than 500 midges were subsampled according to the method of Van Ark & Meiswinkel (1992). Traps were operated at different sites housing varying numbers of horses, sheep, and cattle and compared to control sites with no livestock in the vicinity. For each livestock species (horses, sheep and cattle) two collections were made at five different sites. Traps were hung about 1.8 m above ground level to eliminate height biases. Ten replicates were done at each host from January to March 2009.

The five horse sites were all at stables, housing from five to 25 horses each. All five sites were within a radius of 20 km of Bloemfontein. Except for wild birds and some rodents no other large mammals or livestock were present within 50 m of the stables.

The five sheep sites were all on small-holdings in the Bainsvlei area to the west of Bloemfontein. Individual sites were more than two kilometers apart. At all of these sites sheep were kept in small paddocks, 20 to 50 together, at night. The closest other livestock were more than 50 m from the collection site.

The five cattle sites were mostly dairies and one open field with between 10 and 50 cattle housed at the collection site during the night. The closest other livestock to the sites were more than 100 m away.

The control sites were open grasslands in the area with only a few small trees. The fields would, however, accommodate rodents and birds but the closest livestock or bigger mammals were more than 1 km away.

Care was taken not to select areas with more than one host within at least 50 m of another. The traps were put up at random, according to availability and access to the trapping sites from January to March 2009. Due to the distance between sites and the availability of traps it was not possible to sample all the various sites and hosts in a single night. To minimize the environmental impact, an effort was made not to collect midges during evenings with strong wind or rainfall.

Two-tailed paired t-tests were used to compare the numbers collected at the various sites using GraphPad InStat Version 3. Species diversity at each site was calculated with the Shannon Weiner index, which describes the evenness in distribution of species abundances taking sample size into account. Evenness in distribution of species abundance as determined at the different sites was compared using linear regression GraphPad InStat Version 3.

5.3 Results

The number of *Culicoides* species identified in the ten collections made at each site are shown in Table 5.3.1. From January to March 2009, 26 765 *Culicoides* midges belonging to nine species was the total in 40 collections made at 20 locations over 12 nights.

The number of *Culicoides* midges per collection varied from zero, at the control sites, to more than 2 000 at the horse sites (Table 5.3.1). Significantly higher numbers of *Culicoides* were collected near horses ($P < 0.001$), sheep ($P < 0.001$) and cattle ($P = 0.006$) compared to collections at the control site. The midges in the 10 collections made at the five horse sites accounted for more than 50% of those collected (Table 5.3.1). The average numbers collected at the horse sites (1 357.5) were significantly higher than those collected at the sheep (707.2) ($P = 0.038$) and cattle (479.3) ($P = 0.002$) sites. No significant differences ($P = 0235$) were found in the numbers collected at the sheep and cattle sites.

At all livestock sites and the control sites at least seven species were collected. All species at the control site were also collected in at least one or more of the collections made at livestock (Table 3.5.1). Variations in species richness and species diversity between treatments were the result of single specimens of some species which were collected on only a few trapping occasions (Table 5.3.1).

Table 5.3.1 A summary of *Culicoides* midges collected with FS light traps operated at different livestock hosts in the Bloemfontein area. A total of ten collection were made at each location between January and March 2009

Trap		**Horses**	**Sheep**	**Cattle**	**None**
No. of species collected		7	8	7	8
Total *Culicoides* collected (%)		13 575 (50.7)	7 072 (26.4)	4 793 (17.9)	1 325 (5.0)
Mean collection size		1 357.5	707.2	479.3	132.5
Range in collection size		730-2 460	394-1 145	121-970	2-388
Shannon-Weiner Index		1.47	0.86	0.62	1.20
C. imicola					
	Mean collected (%)	706.3 (52.0)	580.3 (82.1)	419.3 (87.5)	3.4 (2.6)
	Range in collection size	360 – 1035	326 – 1115	108 – 860	0 – 10
C. leucostictus					
	Mean collected (%)	320.0 (23.6)	41.8 (5.9)	15.8 (3.3)	76.0 (57.5)
	Range in collection size	20 – 1 040	5 – 130	4 – 25	0 – 236
C. pycnostictus					
	Mean collected (%)	201.3 (14.8)	51.5 (7.3)	13.5 (2.8)	48.9 (36.9)
	Range in collection size	10 – 700	5 – 150	4 - 20	2 – 140
C. similis					
	Mean collected (%)	20.0 (14.8)	2.5 (0.4)		
	Range in collection size	0 – 80	0 – 10		
C. nivosus					
	Mean collected (%)	72.5 (5.3)	11.3 (1.6)	14.3 (3.0)	2.0 (1.5)
	Range in collection size	0 – 240	7 – 15	3 – 35	0 – 8
C. bolitinos					
	Mean collected (%)	13.8 (1.0)	10.0 (1.4)	14.5 (3.0)	1.3 (1.0)
	Range in collection size	0 – 40	6 – 41	1 – 25	0 – 5
C. bedfordi					
	Mean collected (%)	23.8 (1.8)	8.8 (1.2)	1.3 (0.3)	0.2 (0.2)
	Range in collection size	10 – 40	0 – 14	0 – 5	0 – 1
C. neavei					
	Mean collected (%)			0.8 (0.2)	0.6 (0.5)
	Range in collection size			0 – 3	0 – 5
C. zuluensis					
	Mean collected (%)		1.3 (0.2)		0.1 (7.4)
	Range in collection size		0 – 5		0 - 1

A total of 17 092 *C. imicola* were collected during the 40 collections and this was the most abundant species found near the three selected livestock (Table 5.3.1). The mean number collected varied from zero in the absence of livestock to 706.3 near horses (Table 5.3.1). The mean number (706.3) of *C. imicola* collected at the horses did not differ significantly from the sheep (580.3) (P = 0.686) or cattle (419.3) (P = 0.343), but was significantly higher (P = 0.006) than at the control sites. The mean number of *C. imicola* collected at sheep (580.3) was not significantly higher than at the cattle (419.3) (P = 0.686), but was significantly more than at the control sites (3.4) (P= 0.006). The mean number of *C. imicola* collected at the cattle site was significantly higher (P= 0.006) than at the control site. The relative abundance of *C. imicola* at the livestock sites ranged from 52.0% at the horses to 87.5% at the cattle sites (Table 5.3.1). At the control site it was only the third most abundant species and represented only 3.4% of all the *Culicoides* midges collected (Table 5.3.1).

The second most abundant *Culicoides* species was *C. leucostictus.* A total of 4 535 *C. leucostictus* were present in the 40 collections. It was the most abundant species (57.5%) at the control sites (Table 5.3.1). The mean number of *C. leucostictus* varied from 320.0 near horses to 15.8 collected near cattle (Table 5.3.1). No significant differences were recorded between the mean number of *C. leucostictus* at the three hosts or in their absence. Significant differences were recorded between the proportions of *C. leucostictus* collected near horses (23.6%), sheep (5.9%), cattle (3.3%) and the control sites (57.5%) (P < 0.001), with the exception of the proportions between the sheep and cattle sites (P = 0.054).

A total of 3 152 *C. pycnostictus,* the second most abundant species at the control site, were found in the 40 collections (Table 5.3.1). The mean number of *C. pycnostictus* varied from 13.5 near cattle to 201.3 collected near horses (Table 5.3.1). No significant differences were seen in the mean number of *C. pycnostictus* at the three hosts or in their absence. Significant differences were recorded between the proportions of *C. pycnostictus* collected near horses (14.8%), sheep (7.3%), cattle (2.8%) and the control sites (36.9%) (P < 0.001).

Despite a significant variation in the mean numbers collected a strong linear regression in the species composition was found between the various sites. Based on linear regression the species composition at the cattle site was nearly identical (R^2 =

99.5%) to that of the sheep sites. Strong linear regressions was also found between the cattle and horse sites (R^2 = 81.2%) and the horse and sheep sites (R^2 = 84.3). No linear regression in species composition could, however, be found between the control site and the horse (R^2 = 6.0%), sheep (R^2 = 1.2%) or cattle sites (R^2 = 2.6%).

5.4 Discussion

The highest mean numbers per trap per night (1 357.5) were recorded in the ten collections made at the five horse sites. The mean number of *Culicoides* midges collected at the horse sites was almost double that of the sheep (707.2) and cattle (479.3) sites. The lowest mean number (132.5) was recorded at the control sites. Since these sites were relatively far apart and not sampled on the same nights differences may partly be due to climatic and other environmental factors at the sites. The fact that five different sites were sampled per host and that an effort was made to collect only on nights with comparable conditions should, however, minimize the impact of climate on the results.

Although significantly larger numbers of *Culicoides* were collected near horses the results may have been influenced by environmental factors at the stables. At all the stables, the owners made an effort to irrigate lawns and more trees had been planted and also occurred naturally in these areas when compared to the cattle, sheep and control sites. This is, however, standard practice at most horse stables. The higher abundance of trees ensured a larger population of roosting birds that may explain the higher numbers of *C. leucostictus* and *C. pycnostictus* captured at the stables. Although the total numbers of *Culicoides* collected varied greatly between the sites the mean number and range in collection size of *C. imicola* collected near horses and sheep did not differ significantly. It therefore seems that the big number of midges collected near the horses can rather be attributed to larger numbers of *C. leucostictus* and *C. pycnostictus* than to the presence of large numbers of *C. imicola.*

As was found for most areas in South Africa (Venter *et al*., 1996; Meiswinkel *et al*., 2004) *C. imicola* is the most abundant species at all sites where livestock/mammals are present. *Culicoides* midges at the control sites indicate the constant presence of midges in the environment, mostly actively seeking livestock hosts and then aggregating in large numbers around such hosts for feeding. This indicates a large

dispersal of midges, occurring even some kilometers from the nearest livestock and potential breeding site. The smaller numbers of midges, and especially *C. imicola,* in the absence of livestock is supported by the decrease in *Culicoides* numbers as the light trap is moved away from livestock (Chapter 3). This attraction of *Culicoides* midges, and especially *C. imicola,* will increase the vector capacity of *Culicoides* for the transmission of livestock viruses.

The high linear correlation found in species composition at the livestock sites indicates that several *Culicoides* species may be attracted to livestock. Due to this high linear regression it was not possible to determine a differential attraction of species to horse, cattle and sheep. The low correlation in species composition found between the livestock and the control sites, however, confirms the host preference of *C. imicola* for large mammals.

Potentially all these species can play a role in transmission of viruses and other pathogens to various animals. Several studies have emphasized the different results for abundance determined by light traps compared to biting rates on animals and other non-attractant collection methods (Carpenter *et al*., 2008; Gerry *et al*., 2009). Some of the species found at livestock in the present study are considered to be predominantly bird feeders, *e.g. C. leucostictus* and *C. pycnostictus*. Due to their wide distribution in South Africa and relative abundance near livestock (Nevill *et al.,* 1992a; Meiswinkel *et al*., 2004) and proven oral susceptibly for AHSV and BTV (Venter *et al.*, 2009; 2011) coupled to virus isolations (Nevill *et al.,* 1992b; Meiswinkel *et al*., 2004) from field collected specimens, the role of bird-feeding midges as potential vectors of these livestock viruses cannot be excluded.

Numbers of animals at a collection site will influence the number of midges collected (Garcia-Saenz *et al.,* 2011). This study highlights one of the many factors that can influence light trap collections and the problems in comparing light trap results between different sites and trapping occasions. It also indicates that the interpretation of light trap results in risk determination must be closely linked to the biology of the *Culicoides* species involved.

The central Free State should be considered just as important an area as the endemic regions for disease transmission during favorable times of the year. This also shows

that *C. imicola* is not the only species occurring near all three these hosts, and that other midge species should also be considered when looking at the spread of disease.

5.5 References

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S. & Mellor, P. 2008. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector? *Journal of Applied Ecology*, **45**, 1237-1245.

Garcia-Saenz, A., McCarter, P. & Baylis, M. 2011. The influence of host number on the attraction of biting midges, *Culicoides* spp., to light traps. *Medical and Veterinary Entomology*, **25**, 113-115.

Gerry, A.C., Sarto I Monteys, V., Moreno Vidal, J.O., Francino, O. & Mullens, B.A., 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in northeastern Spain in relation to midge capture using UV light and carbon dioxide-baited traps. *Journal of Medical Entomology*, **46**, 615-624.

Labuschagne, K., Gerber, L.J., Espie, I. & Carpenter, S. 2007. *Culicoides* biting midges at the National Zoological Gardens of South Africa. *Onderstepoort Journal of Veterinary Research*, **74**, 343-347.

Meiswinkel, R. & Braack, L.E.O. 1994. African horsesickness epidemiology: five species of *Culicoides* (Diptera: Ceratopogonidae) collected live behind the ears and at the dung of the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, **61**, 155-170.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: *Infectious Diseases of Livestock* (ed. by J.A.W. Coetzer and R.C. Tustin), pp. 93-136. Oxford University Press, Cape Town.

Mullens, B.A., Gerry, A.C., Lysyk, T.J. & Schmidtmann, E.T. 2004. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpretating laboratory data in a field context. *Veterinaria Italiana*, **40**, 160-166.

Nevill, E.M. & Anderson, D. 1972. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and light trap catches. *Onderstepoort Journal of Veterinary Research*, **39**, 147-152.

Nevill, E.M., Erasmus, B.J. & Venter, G.J. 1992a. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae). In *Bluetongue, African horse sickness and related orbiviruses Proceedings of the 2nd International Symposium* (ed. by T.E.Walton and B.I. Osburn), pp. 314-319. CRC Press, Boca Raton, Florida.

Nevill, E.M., Venter, G.J. & Edwardes, M. 1992b. Potential *Culicoides* vectors of livestock orbivirus. In *Bluetongue, African horse sickness and related orbiviruses Proceedings of the 2nd International Symposium* (ed. by T.E. Walton and B.I. Osburn), pp. 306-313. CRC Press, Boca Raton, Florida.

Nevill, E.M., Venter, G.J., Edwardes, M., Pajor, I.T.P., Meiswinkel, R. & Van Gas, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **55**, 101-106.

Van Ark, H. & Meiswinkel, R. 1992. Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **59**, 183-189.

Venter, G.J., Nevill, E.M. & Van der Linde, T.C. 1996. Geographical distribution and relative abundance of stock associated *Culicoides* species (Diptera: Ceratopogonidae) in southern Africa, in relation to their potential as viral vectors. *Onderstepoort Journal of Veterinary Research*, **63**, 25-38.

Venter, G. J., Wright, I. M., Del Rio, R., Lucientes, J. & Miranda, M.A. 2011. The susceptibility of *Culicoides imicola* and other South African livestock-associated *Culicoides* species to infection with bluetongue virus serotype 8. *Medical and Veterinary Entomology*, **25**, 320-326.

Venter, G.J., Wright, I.M., van der Linde, T.C. & Paweska, J.T. 2009. The oral susceptibility of South African field populations of *Culicoides* to African horse sickness virus. *Medical and Veterinary Entomology*, **23**, 367–378.

6. BREEDING AND OVIPOSITION SITE PREFERENCES OF *C. IMICOLA* AND OTHER LIVESTOCK-ASSOCIATED *CULICOIDES* MIDGES

6.1 Introduction

Culicoides species can utilize a vast number of diverse breeding sites, including the soil-water interface, rock crevices, dung, tree-holes, rotting fruits and vegetation (Nevill, 1967; Meiswinkel *et al*., 2004). Potentially these can be any moist substrate containing organic material in which larvae can tunnel and feed (Nevill, 1967). Most *Culicoides* species are specialist breeders and will only utilize a specific type of substrate (Nevill, 1967).

Culicoides midges are both small in size and have a large variety of breeding sites, the combination of which makes it very difficult to investigate their oviposition preferences (Carpenter *et al.*, 2001). Ceratopogonidae utilize a wide range of exogenous cues to determine suitable oviposition sites and these can include colour, optical density, substrate texture and various chemical stimuli (Carpenter *et al.*, 2001; Borkent, 2005).

Several studies have concentrated on *Culicoides* species that utilize dung pats of large herbivores as breeding sites (Dyce & Marshall, 1988; Meiswnkel & Braack, 1994; Nevill *et al.*, 2007; 2009). The development of *Culicoides* midges from the dung of African game animals is well established (Dyce & Marshall, 1988). Species have been found to breed in the dung of elephant (*Loxodonta africana*), buffalo (*Syncerus caffer*) and zebra (*Equus burchelli*) (Meiswinkel, 1992; Meiswinkel & Braack, 1994; Nevill *et al.*, 1992) while *C. bolitinos* has been reared from the dung of buffalo and domesticated cattle (Dyce & Marshall, 1988; Meiswinkel, 1989).

In South Africa Pajor (1987) was able to collect more than 100 *Culicoides* midges in a newly developed tent-type emergence trap per day per square meter. Also in South Africa Nevill *et al*. (1988) using the same trapping system collected 1601 *Culicoides* in 10 emergence traps over eleven days in irrigated pastures, and furthermore collected *Culicoides* from old moist horse and cow dung. South African livestock-associated *Culicoides* species found in the survey of Nevill *et al.* (1988) were *C.*

gulbenkiani, *C. magnus* and *C. zuluensis. Culicoides gulbenkiani* larval habitats were found to be irrigated pastures grazed by cattle and horses, the substrate was rather dry to slightly moist and rich in decomposed plant matter (Nevill *et al*., 1988). *Culicoides magnus* habitats were sandier to silt-like, waterlogged or almost waterlogged soils with plant cover, decomposed to rotting plant material or dissolved raw manure (Nevill *et al*., 1988). *Culicoides zuluensis* also preferred a wet almost waterlogged, organically rich habitat (Nevill *et al*., 1988).

Despite its relative abundance and wide geographical distribution, relatively little is known about the biology of *C. imicola*. It was found to breed in dry as well as wet kikuyu (*Pennisetum clandestinum)* paddocks, especially where horses were kept (Nevill *et al*., 1988). The breeding sites of *C. imicola* have been defined as being soils that are organically enriched but not waterlogged (Meiswinkel, 1989; Meiswinkel *et al*., 2004). This species was found to predominate in moisture retentive clay soils which might be unvegetated or covered in short grass as in pastures and also in wet, organically enriched kikuyu pastures (Meiswinkel, 1989; Meiswinkel *et al*., 2004).

Identifying and determining preferred breeding substrates of *C. imicola* and other potential vector species may in future contribute to the integrated control of the midges and midge-borne diseases. In the present study an effort was made to determine some of the factors, which might influence the oviposition site preferences in *C. imicola* and other livestock *Culicoides* species. In addition the egg batch sizes of *C. imicola* were determined and compared to those of other *Culicoides* species.

6.2 Material and Methods

6.2.1 Preference for oviposition substrate

Live midges were collected with the FS light traps by replacing the buffer solution in the collection beaker with a piece of crumpled up tissue paper (Venter *et al.*, 1998). Collections were made at the height of the *Culicoides* season during February and March of 2010, at horse stables (29˚03’48’’S, 26˚06’34’’E), housing six horses, near Bloemfontein in the central Free State (Figure 1, Chapter 4). The crumpled paper served to protect midges from the constant down draught of the light trap fan. Traps were run from dusk to dawn and the next morning the captured midges were immobilized by placing them in a walk-in refrigerator at 5°C.

The midges were sorted to species level inside the refrigerator. Only nulliparous females were used and fed in the laboratory to eliminate the discrepancies in bloodmeal size between naturally fed females and those fed using cotton wool pledgets. Nulliparous *C. imicola* females were sorted and kept in cardboard cups, 50 per cup for 24h with a 10% sucrose solution on cotton pads as nourishment. Before blood feeding, midges were starved for 24h and then fed on fresh sheep's blood on a cotton wool pledget placed on the gauze covering the cardboard cup according to the method of Venter *et al.* (2005). The blood was heated to 37°C in a cup submerged in a water bath before transferring the cotton wool with blood to the cardboard cup. After about an hour of feeding the midges were again chilled at 5°C and all the blood-engorged females sorted out.

The oviposition options included filter paper (65 mm diameter) kept on wet cotton wool in a petri dish (65 mm diameter) and moistened daily with extracts made from cattle dung, horse dung, sheep dung, grass, soil and distilled water. Extracts were prepared by grinding the substrate to a pulp and adding water to obtain a slurry. The mixtures were left for three days then filtered through a rough mesh to remove large pieces of debris. The six different oviposition choices were presented to the midges in a single container, 20 x 20 x 20 cm with these choices offered in the bottom of a mesh-covered box. All sides were covered with the fine mesh except for the bottom where the options were offered through six holes in the hardboard footplate.

The midges and subsequent eggs were kept at a 12L:12D photoperiod at 75% RH between 20 to 25°C and fed daily on a 10% sucrose solution on cotton wool pledgets during the time allowed for oviposition. After two weeks the eggs were counted and the preferred substrate identified. Three replicates of 30 midges were done.

6.2.2 The influence of relative humidity on oviposition

Collection of live midges and the blood feeding of field-collected midges was done as described above. Thirty blood-engorged *C. imicola* females were placed in one of three containers, each with a different percentage of relative humidity (RH), 50%, 75% and 95%, obtained by using different salt solutions. Moist filter paper was provided as oviposition substrate. Two weeks were allowed after which the eggs were counted and the influence of RH on oviposition determined. The blood-engorged

females were kept at a 12L:12D photoperiod between 20 to 25°C. Three replicates of the 30 midges were done.

6.2.3 Numbers of eggs per midge species

Gravid *Culicoides* females collected in the samples as described in chapter four, were preserved in 70% ethanol for dissection to determine the number of eggs per female. Gravid females were sampled when available during the peak in midge numbers. Dissection, using fine needles, and counting was done under a stereo microscope. Where possible at least 10 females of each species were dissected. Means of the number of eggs per female were calculated for each species.

6.2.4 Sampling of possible larval habitats

To determine potential larval habitats, samples of dung from large herbivores were collected and placed in emergence boxes at 25°C and 70% RH for three to four weeks (Nevill *et al.*, 2007). The boxes were 25 by 35 cm, fitted with a non-transparent funnel with a cup covered with gauze to collect emerging midges. The light coming from the opening at the tip of the funnel was the only light entering the box. Ten samples each (~2 L) were, collected during February and March 2009, comprising dung of the African elephant (*Loxodonta africana*), zebra (*Equus burchelli*), white rhino (*Ceratotherium simum*), horse and bovine. Game samples were collected at the Bloemfontein Zoo, situated in the city center. The dung of the previous day, after having been left overnight in the enclosures, was collected before the cages were cleaned each morning. This was done to determine which *Culicoides* species, if any, could be reared from the samples.

An additional ten samples each (~2 L) of horse and cattle dung collected from a field in the Bloemfontein area were also incubated. An effort was made to collect samples not more than one day old.

Tent type emergence traps as described by Pajor (1987), made of gauze, with a base size of 1 m by 1 m were used to sample possible breeding sites in the field. The trap funneled upwards towards a collection bottle filled with a 5% Savlon solution to trap the emerging midges. These traps were placed over possible breeding sites such as moist grass patches and other wet organic matter in an area surrounding horse stables

during February to May 2009 when large light trap catches were being made during the night (Chapter 4). The three traps were put up and emptied after one week and moved to a new location.

Nonparametric Repeated Measures ANOVA (Friedman Test) were used to differentiate between treatments.

6.3 Results

6.3.1 Preference for oviposition substrate

A total of 1 383 eggs were counted after the two-week incubation period of the three replicates. The average number of eggs per female ranged from 1.2 (horse dung) to 3.6 (grass) (Table 6.3.1). Most eggs (23.2%) were deposited on the filter paper treated with the kikuyu extract (Table 6.3.1). The second highest number was collected from the filter paper treated with the clean water and the least number of eggs were collected from the filter paper treated with horse dung extract. Taking into account the substantial variation between replicates, these differences were considered as not statistically significant ($P = 0.162$).

Table 6.3.1 The number of eggs deposited by 30 *Culicoides imicola* females in three replicates on different oviposition substrates

Replicate	Clean Water	Cattle dung	Horse dung	Grass	Sheep dung	Soil
1	75	75	33	81	42	87
2	126	54	27	177	159	54
3	87	102	45	63	45	51
Total	288	231	105	321	246	192
Mean	96	77	35	107	82	64
(%)	(20.8)	(16.7)	(7.6)	(23.2)	(17.8)	(13.9)
No of egg/per female	3.2	2.6	1.2	3.6	2.7	2.1

6.3.2 Preference of humidity for oviposition

A total of 879 eggs were found after the two-week incubation period for the three replicates. The midges deposited more eggs at 75% RH (435) than at 50% RH (42) (Table 6.3.2). The mean eggs per female ranged from 0.47 at 50% RH to 4.8 at 75% RH (Table 6.3.2). Significantly more eggs were collected on average at 75% RH ($P < 0.001$) and 95% RH ($P < 0.001$) than at 50% RH. However, when taking into

account the substantial variation between replicates these differences were not significant ($P = 0.194$).

Table 6.3.2 The number of eggs deposited by 30 *Culicoides imicola* midges over three replicates with different oviposition humidities

Replicate	50% RH	75% RH	95% RH
1	24	108	27
2	3	114	312
3	15	213	63
Total	42	435	402
Mean	14	145	134
(%)	(4.8)	(49.5)	(45.7)
No of egg/per female	0.4	4.8	4.5

6.3.3 Numbers of eggs per midge species

A total of 46 gravid females representing nine species were dissected and the numbers of eggs determined. The mean number of eggs per single female ranged from 38 in *C. imicola* to 136 in *C. leucostictus* (Table 6.3.3). There was no significant difference between the number of eggs removed from *C. imicola* on average (46 eggs per female) and *C. bolitinos* (38 eggs per female). This is a relatively small number of eggs compared to other species, especially *C. leucostictus* (136 eggs) and *C. nivosus* (127 eggs per female midge) (Table 6.3.3).

Table 6.3.3 The number of eggs dissected from individual gravid field collected *Culicoides* species

Species	Number of females dissected	Total	Range	Average
C. bolitinos	11	418	27-56	38
C. imicola	10	460	34-62	46
C. pycnostictus	9	999	71-163	111
C. nivosus	7	889	92-173	127
C. leucostictus	5	680	98-197	136
C. exspectator	1	116	116	116
C. magnus	1	100	100	100
C. similis	1	87	87	87
C. zuluensis	1	86	86	86

6.3.4 Sampling of possible larval habitats

No *Culicoides* emerged from the ten samples of elephant, zebra and rhino dung, that were collected from the Bloemfontein Zoo or the additional ten samples of horse and cattle dung collected from the field. Other Dipteran insects were collected from these samples.

No *Culicoides* were collected from the three emergence traps, put up and emptied on a weekly basis from February to May 2009. Other Dipteran insects were collected in these traps.

6.4 Discussion

6.4.1 Preference for oviposition substrate

No significant differences were observed between the different substrates evaluated for oviposition. This was in contradiction to the preliminary studies of Boikanyo & Venter (2009) who indicated that filter paper moistened with extracts of horse dung were preferred for oviposition. In the present study eggs were deposited randomly on all six substrates evaluated and the availability of a moist substrate seems to have been the only criterion for oviposition. The large numbers of *C. imicola* usually collected from areas with livestock led to the expectation that it would also play a role in oviposition preferences.

6.4.2 Preference of humidity for oviposition

Similarly results obtained for the oviposition substrates evaluated revealed no significant differences in the number of eggs deposited between the different relative humidity levels. The substantial variations in the individual numbers of eggs collected between replicates may indicate that a larger sample of midges or more replicates might have led to more significant results.

The fecundity of *C. imicola* in the present study was low in comparison to what is known for this species. Nevill (1967) found 69 eggs per female and Braverman & Linney (1994) 53–65 eggs per female. This very low fecundity could have many underlying causes. An understanding of the cues for oviposition would improve the yield and the accuracy of the data. Venter *et al*. (2005) demonstrated that a smaller

blood meal is taken up using the cotton wool pledget than membrane feeding, leading to smaller egg batches. High mortality in the midges under laboratory conditions could have had a great influence on these results and this has to be monitored closely in these experiments.

6.4.3 Numbers of eggs per midge species

The numbers of midge eggs dissected from the different midge species coincides with the numbers dissected in previous studies (Nevill, 1967). The mean number of eggs per *C. imicola* female was much higher in field collected midges than those fed artificially in laboratory experiments. It was not surprising that the number of eggs dissected from *C. imicola* and *C. bolitinos* did not differ significantly. These species are of similar size and both belong to the sub genus *Avaritia* (Meiswinkel, 1989).

The differences in batch size of different species needs to be linked to the size of the midge and the length of the gonotrophic and life cycles of the *Culicoides* species involved.

6.4.4 Sampling of possible larval habitats

The lack of midges collected from the animals in the Zoo can be attributed to a variety of factors, from the artificial diet of the animals to the lack of constant breeding sites in enclosures which are cleaned on a daily basis and also the location of this Zoo in the city centre.

In South Africa *C. bolitinos* was the first *Culicoides* species to be reared from cattle dung (Nevill, 1968). Under laboratory conditions buffalo dung can produce *C. bolitinos* for up to 90 days, especially during cooler months when it does not desiccate as rapidly as during the hot summer months and the disruptive influence of dung beetles and birds is less obvious (Meiswinkel *et al*., 2004). Most of the other *Culicoides* species that are known to be reared from the dung of rhino and zebra have not yet been described (Meiswinkel *et al*., 2004; Nevill *et* al., 2007; 2009).

Culicoides bolitinos and *C. gulbenkiani* were expected to be collected from the dung samples collected from the field. These two species are known to breed in dung and

were found in light trap collections made at the same sites where the dung was sampled.

Further studies to identify the breeding sites of the *Culicoides* should be conducted to characterise these sites more specifically. The methods used during these experiments are an indication of the type of experiment which needs to be done, but the techniques have to be refined and the cues for oviposition determined more carefully. More repetitions should be done and an effort to use gravid females collected in light traps will already improve the reliability of the results.

6.5 References

Boikanyo, S.N.B. & Venter, G.J. 2009. Preliminary studies on oviposition site preferences of *Culicoides imicola* (Diptera: Ceratopogonidae). (Abstract). *Journal of the South African Veterinary Association*, **80**, 135.

Borkent, A. 2005. The biting midges, the Ceratopogonidae (Diptera). In: W.C. Marquardt, W.C., (Ed.) Biology of disease vectors 2nd ed., Elsevier Academic Press, San Diego, pp. 113-126.

Braverman, Y., Linley, J.R., 1994. Fecundity and proportions of gravid females in populations of the bluetongue vector *Culicoides imicola* (Diptera: Ceratopogonidae) and several other species in Israel. *Journal of Medical Entomology*, **31**, 838–843.

Carpenter, S., Mordue, A.J. & Mordue, W. 2001. Oviposition in *Culicoides impunctatus* under laboratory conditions. *Entomologia Experimentalis et Applicata*, **101**, 123–129.

Dyce, A.L. & Marshall, B.D. 1988. An early record of *Culicoides* species (Diptera: Ceratopogonidae) developing in the dung of game animals in southern Africa. *Onderstepoort Journal of Veterinary Research*, **56**, 85-86.

Meiswinkel R., 1989. Afrotropical *Culicoides*: a redescription of *C.* (*Avaritia*) *imicola* Kiefer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C.* (*A.*) *bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, **56**, 23-39.

Meiswinkel, R. 1992. Afrotropical *Culicoides: C. (Avaritia) loxodontis* sp. nov., a new member of the Imicola group (Diptera: Ceratopogonidae) associated with the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, **59**, 145–160.

Meiswinkel, R. & Braack, L.E.O. 1994. African horsesickness epidemiology: five species of *Culicoides* (Diptera: Ceratopogonidae) collected live behind the ears and at the dung of the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, **61**, 155-170.

Meiswinkel, R., Venter, G.J. & Nevill, E.M. 2004. Vectors: *Culicoides* spp. In: *Infectious Diseases of Livestock* (ed. by J.A.W. Coetzer and R.C. Tustin), pp. 93-136. Oxford University Press, Cape Town.

Nevill, E.M., 1967. Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. M.Sc.(Agric) Thesis. University of Pretoria, Pretoria.

Nevill, E.M. 1968. A significant new breeding site of *Culicoides pallidipennis* Carter, Ingram and Macfie (Diptera: Ceratopogonidae). *Journal of the South African Veterinary and Medical Association*, **39**, 61.

Nevill, E.M., Venter, G.J., Edwardes, M., Pajor, I.T.P., Meiswinkel, R. & Van Gas, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **55**, 101-106.

Nevill, E.M., Erasmus, B.J. & Venter, G.J. 1992. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae). In *Bluetongue, African horse sickness and related orbiviruses Proceedings of the 2nd International Symposium* (ed. by T.E. Walton and B.I. Osburn), pp. 314-319. CRC Press, Boca Raton, Florida.

Nevill, H., Venter, G.J., Meiswinkel, R. & Nevill, E.M. 2007. Afrotropical *Culicoides*: Description of the pupae of five species of the Imicola Complex, subgenus Avaritia, Fox, (Diptera: Ceratopogonidae) from South Africa. *Onderstepoort Journal of Veterinary Research*, **74**, 97-114.

Nevill, H., Nevill, E.M. & Venter, G.J. 2009. Description and comparison of the pupae of a further two *Culicoides* (*Avaritia*) species from the dung of large herbivores in South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **76**, 277-284.

Pajor, I.T. 1987. A collapsible, semi-automatic, tent type, emergence trap, suitable for sampling *Culicoides* (Diptera: Ceratopogonidae) from a wide range of habitats. *Onderstepoort Journal of Veterinary Research*, **54**, 99 - 101.

Venter, G.J., Paweska, J.T. van Dijk, A.A., Mellor, P.S. & Tabachnick, W.J. 1998. Vector competence of *Culicoides bolitinos* and *C. imicola* (Diptera: Ceratopogonidae) for South African bluetongue virus serotypes 1, 3 and 4. *Medical and Veterinary Entomology*, **12**, 101-108.

Venter, G.J., Paweska, J.T., Lunt, H., Mellor, P.S. & Carpenter, S. 2005. An alternative method of blood-feeding *Culicoides imicola* and other haematophagous *Culicoides* species for vector competence studies. *Veterinary Parasitology*, **131**, 331-335.

7. CONCLUSION

7.1 *Culicoides* species occurring in the Free State

The four most frequent *Culicoides* species collected were *C. imicola*, *C. leucostictus*, *C. pycnostictus* and *C. bolitinos*. Based on the abundance in light trap collection and frequency of collection the species with the highest vector rating in the present survey were *C. imicola*, *C. pycnostictus* and *C. leucostictus*.

Culicoides imicola

Culicoides imicola (Subgenus: *Avaritia*) has been shown to be the most abundant species of *Culicoides* in South Africa. Because of its wide distribution, strong association with livestock and high abundance, *C. imicola* is rated as the most important vector of AHSV and BTV in South Africa.

Culicoides leucostictus

Culicoides leucostictus has a wide distribution, but it is only abundant in collections made in the presence of poultry and other birds. This species feeds predominantly on birds and poultry and can therefore be a vector of bird-related viruses.

Culicoides pycnostictus

Culicoides pycnostictus feeds mainly on poultry, but may feed on cattle and horses and is one of the most abundant species in the Free State. Its role as a potential vector of orbiviruses is highlighted by the isolation of at least two serotypes of BTV, BTV 2 and 24, from field-collected midges.

Culicoides bolitinos

Culicoides bolitinos is more abundant in the colder high-lying areas, e.g. the eastern Free State. This species is strongly associated with livestock, breeding in the dung of large herbivores and feeding on cattle, sheep and horses. Isolations of AHSV were made from *C. bolitinos.*

7.2 The influence of rainfall on the occurrence and abundance of *Culicoides* midges

Culicoides midges, and especially the larval stages, are extremely dependent on moisture and temperature to exist and flourish. They need the wet marshy areas for breeding areas and to reach high numbers. Rainfall combined with favourable temperatures had the most noticeable impact on the number of midges that occurred in an area. The increased relative humidity and availability of breeding sites during the rainy season lead to a rapid increase in the numbers of *Culicoides* midges that were collected in the light trap collections. High rainfall can also influence the numbers of *C. imicola* negatively and lead to the midge pupae drowning and delay the emergence of the next generation of midges.

7.3 The suitability of light traps as monitoring tool

Light traps are an important and useful tool when monitoring *Culicoides* midges. The importance of the correct interpretation of the sample collected and the understanding of the operation of light traps to optimize and standardise collections is important when comparing data collected. Factors that can directly influence collection size include wind speed, humidity, rainfall, trapping height, trap distance from animals, the abundance of different livestock species and the type of trap used. In order to assess the collections made by the light traps accurately one has to consider that the light trap has vital flaws. These include the collection of less than 0.0001% of the midges in the area, collecting markedly fewer gravid females, freshly bloodfed females and males. The number of midges collected in a light trap is not an accurate indication of the midges biting rate. It is only an indication of the numbers and species of midges in the area. Despite the great number of factors affecting light trap collections, they are still the most effective and reliable method to collect midges and monitor their relative abundance and seasonality.

7.4 Factors influencing the efficacy of light traps

Two of the most important influences on light trap collections as discovered during this research were firstly, the trap type. The trap developed in the Free State seemed to be an exact replicate of the Onderstepoort trap, with the exception of the distance between the light source and the fan. However, the results of the comparisons of these

traps revealed that the Onderstepoort trap collected samples of more five times the number of midges collected by the Free State trap. The overall composition of the collections did not differ from each other, again emphasising the standardisation of trapping methods when comparing data. The second important factor assessed was the trapping distance from the host animals. This had an enormous influence on the collection size, species composition and age grading of the collections. Collections made only five meters from the host animals already showed a marked decrease of the vector species. *Culicoides* midges collected during this survey did not show any preference for a specific light colour; the intensity of the light source seemed to have a greater influence.

7.5 Factors influencing oviposition preferences and larval habitats of *Culicoides* midges

No significant differences were observed between the different substrates evaluated for oviposition. The large numbers of *C. imicola* usually collected from areas with livestock led to the expectation that it will also play a role in oviposition preferences. Similar to results obtained for the oviposition substrates evaluated no significant differences in the number of eggs deposited were observed between the different relative humidities evaluated. Further studies to identify the breeding sites of the *Culicoides* midges should be conducted to determine more specifically the breeding sites.

The diseases spread by *Culicoides* vectors should therefore be controlled continuously in the Free State to protect livestock. As the midges are small and occur in such large numbers and their breeding sites are difficult to specifically determine, the main focus of disease control should remain the vaccination of animals.

7.6 The risk of AHS in the Free State

Culicoides midges were present in most of the light trap collections made in the central Free State. *Culicoides* midges belonging to 20 species were collected over a period of 40 months. The most frequent and abundant species collected was *C. imicola*. This midge species is the most important, confirmed vector of both the African horse sickness virus (AHSV) and the bluetongue virus (BTV). *Culicoides imicola* was collected year round in the light trap collections made near horses and

sheep. The periods of high risk for disease transmission in the central Free State is from February to May, during years with above average rainfall, when the midge population peaks as frequent disease transmission can occur. However, these midges were able to mate, take a bloodmeal and potentially transmit disease throughout the year. The low infection rate limits the transmission of disease when the midge numbers are low, but the risk remains year round. The year round occurrence can also implicate *C. imicola* as an overwintering host for these viruses. This indicates that the central Free State may be more important in the disease cycles in South Africa than initially believed.

ABSTRACT

Culicoides midges are involved in the transmission of a variety of pathogens, the most economically important of these are the orbiviruses that cause African horse sickness (AHSV) and bluetongue (BTV). The identification of vectors of these viruses and monitoring of their occurrence and activity plays an important role in the control measures and disease risk analysis. The primary tool used for monitoring these midges through collection is various models of light traps.

In order to standardise collection data to be comparable between laboratories, a variety of factors that affect the light trap collections were assessed. Comparisons of different light traps (Onderstepoort trap and the Free State trap), the influence of light colour, trapping height and the distance a trap is operated from the host animals were assessed. Comparisons were done using either three replicates of a 4 x 4 or two replicates of a 6 x 6 randomised Latin square design. The most significant variables were the trap type, with the Onderstepoort trap collecting significantly more *Culicoides* than the Free State trap, and the sampling distance from the host animals. The proportion of *C. imicola* (the most frequent species collected) was the highest when collected right next to host animals and decreased rapidly as collections moved further from host animals. Trap height also proved to be an important variable, although no significant differences were observed when collecting midges at two metres to three metres above the ground.

The occurrence, abundance and seasonality of the midges frequenting livestock in the central Free State were also assessed by collecting midges using light traps over a four-year period from April 2007 up to May 2010. Twenty *Culicoides* species were collected, the most abundant and important species was *C. imicola*, a confirmed vector of both the AHSV and BTV. The midges showed a distinct seasonal pattern, but were also collected year round, identifying periods of high risk, as well as a year-round risk of disease transmission. The midge populations almost disappeared when temperatures dropped during the winter months, the build-up and abundance during favourable conditions, however, indicated a high risk for disease transmission.

Culicoides imicola also showed a considerable preference for livestock animals when assessing collections made near horses sheep and cattle as opposed to collections made in the absence of livestock host animals.

An effort was made to identify possible breeding sites of *Culicoides* species. No midges were, however, collected in the tent type traps or the dung and soil samples collected and placed in emergence boxes. This again emphasised the diversity of the midges' breeding habitats and the enormous task still ahead to identify these sites to aid in possible reduction of midge numbers.

Keywords: *Culicoides*, *Culicoides imicola*, light traps, seasonal patterns, host preference, breeding sites.

OPSOMMING

Culicoides muggies is betrokke by die verspreiding van ‘n verskeidenheid patogene, die van meeste ekonomiese belang is behoort aan die familie orbivirusse wat insluit die virusse wat Afrika perdesiekte (AHS) en Bloutong (BT) veroorsaak. Die identifisering van die vektore van die virusse en die monitering van hulle voorkoms en aktiwiteit speel ‘n belangrike rol in die beheer maatreëls en siekte risiko analises. Die primêre monitering van die muggies geskied deur middel van verskeie variasies ligvalle wat opgesit word naby gasheer diere en deur die nag muggies versamel.

Om die ligval versameling te standariseer en die data van verskillende areas en versameling vergelykbaar te maak is ‘n reeks veranderlikes vergelyk. Vergelykings van verskillende ligvalle (Onderstepoort ligval en die Vrystaat ligval), die invloed van ligval kleur, ligval hoogte en die afstand wat ligvalle vanaf die naaste gasheer diere is vergelyk. Vergelykings is gedoen deur middel van die gebruik van drie herhalings van 4 x 4, of twee herhaling van 6 x 6 ewekansige latynse vierkant ontwerp. Die mees beduidende invloed was die soort ligval. Die Onderstepoort ligval het beduidend meer *Culicoides* muggies versamel as die nuut ontwikkelde Vrystaat ligval. Die afstand van die naaste gasheer dier het ook ‘n beduidende invloed op die muggie getalle gehad. Die proporsie *C. imicola* (die volopste spesie versamel) was die hoogste as die ligval reg langs die gasheer diere opgesit is en het baie vining verlaag soos die versamelings verder van die gasheer diere af weg gemaak is. Die ligval hoogte het ook ‘n beduidende invloed gehad: meer *C. imicola* is versamel van twee to drie meter bo die grond as by een en ‘n halwe meter hoog.

Die voorkoms, volopheid en seisoenaliteit van die *Culicoides* muggies wat gasheer diere besoek in die sentrale Vrystaat is bepaal deur monitoring met ligvalle oor ‘n vier jaar periode vanaf April 2007 tot en met Mei 2010. Twintig spesies van *Culicoides* muggies is versamel, waarvan die volopste *C. imicola* was. *Culicoides imicola* is ‘n bevestigde vektor spesie van beide die Afrika perdesiekte virus sowel as die Bloutong virus. Die muggies het ‘n duidelike seisonale voorkoms, maar was wel deur die jaar teenwoordig. Dit dui op tye van hoë risiko vir siekte oordrag tydens voordelige tye van die jaar in die laat somer en herfs maande, sowel as dat die virus deur die jaar in die sentrale Vrystaat kan voorkom en die winter oorleef in die oorlewende muggies.

Die muggie voorkoms het baie verlaag en amper verdwyn tydens die koue wintermaande, maar die opbou van die populasie in die daaropvolgende gunstige maande het gedui op defnitiewe hoë risiko tye vir siekte oordrag.

Culicoides imicola het ook ‘n beduidende voorkeur gewys vir grootvee in versameling wat gemaak is naby perde, skape en beeste en vergelyk is met vangste wat in die afwesigheid van vee gemaak is.

Die muggies se broei en onvolwasse habitat is ook geëvalueer om die moontlikheid van beheer in die areas te ondersoek. Daar was egter geen muggies versamel uit die onvolwasse habitats wat ondersoek is nie. Dit dui weer op die verskeidenheid moontlike broei en onvolwasse habitats en die omvang van die taak indien die areas as ‘n moontlike fokus vir die beheer van die muggies of self ‘n verlaging van muggie getalle sou gebruik word.

Sleutelwoorde: *Culicoides*, *Culicoides imicola*, ligvalle, seisoenaliteit, gasheer voorkeur, broei en onvolwasse habitats.