A STUDY OF THE *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) VECTORS OF AFRICAN HORSE SICKNESS TO ENHANCE CURRENT PRACTICAL CONTROL MEASURES AND RESEARCH METHODS.

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I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other University.

Alexander Byron Jenkins
May 2008

I hereby release this thesis for examination in my capacity as supervisor.

Ms M B Young
May 2008

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PREFACE

African horse sickness (AHS) virus causes a non-contagious, infectious disease of equids. It is epizootic to sub-Saharan Africa and parts of the Middle East. Due to climatic change it may recently have spread to Europe. It is vectored by species of *Culicoides* midges (Diptera: Ceratopogonidae) and most importantly by the two Avarita species of *C. imicola* Keiffer and *C. bolitinos* Meiswinkel. Literature pertaining to the study and research of the virus, the disease and the vectors is reviewed. Models for prediction of future possible outbreaks as well as control strategies are discussed, as are reservoir hosts and control measures in South Africa.

A new, electrical, revolving, trap was designed to test the preference of small nocturnal flying insects for light colour and source. Three commercially available, propane combusting, pheromone baited traps were tested at a farm in KwaZulu-Natal (KZN) to determine their efficacy at out-trapping *Culicoides* midges. Identification of breeding sites of AHS vectors with tent traps at 90 locations in the KZN-midlands indicated that sites with increasing ground moisture, increasing incident radiation and increasing wetness duration were found to positively increase the number of midges collected from them. Site preferences of adult *Culicoides* midges were also examined to identify focal spots for vector control. Five similar regions across five farms were sampled over one year. Midges were found to frequent dung heaps and the interior of stable blocks significantly more than any other site, especially during July when temperatures are at their lowest and midges find shelter, warmth and food in these places. Light colour and source preference testing has been conducted for *C. brevitarsus* in Australia but has not been performed in South Africa. Data from the Australian trials show an increased affinity for light from Light Emitting Diodes (LEDs) rather than incandescent light (the Australian Standard). A new trap was used to compare midge attractiveness to fluorescent and LED light sources as well as the colours: white, green and ultraviolet (UV). Results show a very high affinity for UV light. Catches from white and green light were not found to differ significantly and the interaction between light colour and source was not found to be significant. Possible trap development and action thresholds are discussed as interventions in the control of AHS.

CHAPTER 1: A REVIEW OF LITERATURE PERTAINING TO AFRICAN HORSE SICKNESS AND THE VECTOR *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE).

A. B. JENKINS

1.1 Abstract

African horse sickness virus causes a non-contagious, infectious disease of equids. It is epizootic to sub-Saharan Africa and parts of the Middle East. The epizootics caused by the virus have caused widespread devastation amongst equids worldwide. Fortunately no epizootic has lasted more than 5 years outside of sub-Saharan Africa. It is vectored by species of *Culicoides* midges (Diptera: Ceratopogonidae) and most importantly by the two Avarita species of *C. imicola* Keiffer and *C. bolitinos* Meiswinkel. The literature pertaining to the study and research of the virus, the disease and the vectors is reviewed. Models allowing prediction of future possible outbreaks as well as details of control strategies and findings of researchers are presented and discussed. The virus needs a long term reservoir host in which to overwinter and various theories are discussed. Control measures in South Africa are suggested so that outbreaks of the disease can be reduced.

1.2 African Horse Sickness

1.2.1 History

African horse sickness (AHS) is a non-contagious arthropod borne disease of equids (Meiswinkel & Paweska, 2003). It is one of the most lethal of the infectious horse diseases with mortality rates of up to 95% under laboratory conditions (Mellor *et al*., 2000; Meiswinkel & Paweska, 2003). The disease itself was first described in 1327 in Yemen by Moule in an Arabian document entitled “Le Kitâb El-Akouâl El-Kafiah Wa El Chafiâh” (Henning, 1932). In 1569 during his journey to East Africa, Father Monclaro noted a disease that can be identified as African horse sickness that ravaged horses imported from India to the region (Henning, 1932). In South Africa, it was first recognized in 1717, 60 years after the initial introduction of horses by settlers in 1657 (Mellor & Hamblin, 2004). In 1719 the first plague occurred in the Cape of Good Hope and nearly 1700 horses succumbed to the disease known as ‘perdesiekte’ (Henning, 1932). Severe outbreaks occurred in 1780, 1801, 1839, 1854-1855, 1857, 1862, and 1891. The outbreak in 1854-5 was the most virulent outbreak on record where some 70 000 horses died. This was about 40% of the total population of horses in the Cape of Good Hope at the time (Henning, 1932). For many years the disease was confused with diseases like anthrax, malaria, encephalosis, biliary fever, piroplasmosis and heartwater, but was finally confirmed separately as a viral disease by M’Fadyean, Theiler and Nocard in the years 1900 and 1901 (Henning, 1932). In 1903, it was proposed by Pitchford and Theiler (Henning, 1932) that the disease was transmitted by biting insects and in 1944 the midge, *Culicoides imicola,* was reported by Du Toit (1944) as being the vector of both Bluetongue and African horse sickness.

1.2.2 Animal species susceptible

African horse sickness is caused by the African horse sickness virus (AHSV). In horses the virus is the most virulent with mortality rates of between 70 and 95%. Mules are less susceptible with mortality rates of between 50 and 70%. African donkeys and zebra (E*quus burchelli*) are highly resistant to the virus and only show signs of a slight fever when artificially inoculated with the virus. When spleen tissue taken from a donkey was homogenized and injected into a pony, a fatal attack of HS

occurred (Mellor & Hamblin, 2004). Some resistance has been noted (Hamblin *et al*., 1991) in Spain to AHSV serotype 4 and in North and West Africa where long lineages of horses date back to at least 2000BC (Bourdin, 1973, cited in Coëtzer & Erasmus, 1994). Dogs are also affected fatally by African horse sickness virus (Henning, 1932; Braverman & Chizov-Ginzburg, 1995) and it is possible to transmit the virus from dog to dog and from dog to horse. All reported clinical cases thus far have occurred after dogs have been fed infected horse meat (Coëtzer & Erasmus, 1994) It was shown by Braverman & Chizov-Ginzburg (1995) that dogs are highly unlikely to act as a reservoir to the disease as none of their 1123 replicates were actually bitten by midges, thus proving that midges seldom attack them.

1.2.3. Clinical Forms of AHS

There are 4 forms in which AHS can be manifested in the host.

The subclinical form, also known as horse sickness fever, is the least virulent of the four forms. Affected animals have a mild to moderate fever accompanied by oedemia of the supraorbital fossae. Animals may appear depressed and exhibit a slight fever but do not go off their food and will continue eating. Animals may exhibit this form of AHS if they have some kind of immunity to the virus, either through vaccination or recovery from a prior infection, or if they are simply challenged with one of the less virulent strains of the virus. It is this form that is only ever exhibited by African donkeys and zebras and is never fatal Coëtzer & Erasmus, 1994; Barnard, 1998). In a study done on African donkeys by Hamblin *et al.* (1998), the only gross pathological changes to the animals were an increase in fluid accumulation in the serosal lined compartments and petechial and ecchymotic haemorrhages on the left hepatic arch.

The acute or cardiac form of AHS is far more severe with mortality rates rising to around 50%. Also known as ´dikkop´or 'thick head' in South Africa, animals show signs of a high fever of 39°-40°C that may last for several weeks. They are depressed and their food intake drops. They often have severe colic and may have oedema of the head, neck, chest, and supraorbital fossae. They exhibit petechial haemorrhaging and ecchymotic haemorrhaging on the bottom of the tongue (Coëtzer & Erasmus, 1994).

The mixed form is less virulent than the pulmonary form of which it is comprised and is the most common form of AHS, although often not clinically diagnosed as such due to the mix of symptoms. Death tolls from this form are about 70% (Coëtzer & Erasmus, 1994).

The diagnosis of an animal with the pulmonary or peracute form is a very serious one indeed. 'Dunkop', as it is known in South Africa, has very high mortality rates. Only about 5% of horses recover. Death can occur so rapidly that an owner may not notice the animal is sick (Young, *pers. comm*.) It is the only form seen in dogs and is fatal to them. In horses the symptoms are depression and a high fever (39-41℃) accompanied by coughing spasms, respiratory distress and severe dyspnoea. The animal may sweat severely and extend its neck and head. Animals are often recumbent and expel frothy liquid from the nares before death (Coëtzer & Erasmus, 1994).

1.3 African Horse Sickness Virus

1.3.1 Overview

The virus is an arthropod borne virus, meaning that it infects a biting insect vector, multiplies in it and then is transmitted to the equid host. It is thus called an arbovirus (arthropod-(ar)-borne (bo)-virus) and is in the family Reoviridae (Kettle, 1984). It is closely related in structure to the Blue Tongue Virus (BTV) of sheep and the Equine Encephalosis Virus (EEV) in equids. It is made up of structural and non structural proteins of which there are twelve in total (Grubham & Lewis, 1992). Figure 1.1 shows a model of Blue Tongue virus. Nine different serotypes of the virus have been identified. There is no evidence of intratypic variation, meaning that serotypes can not cross with one another (Coëtzer & Erasmus, 1994).

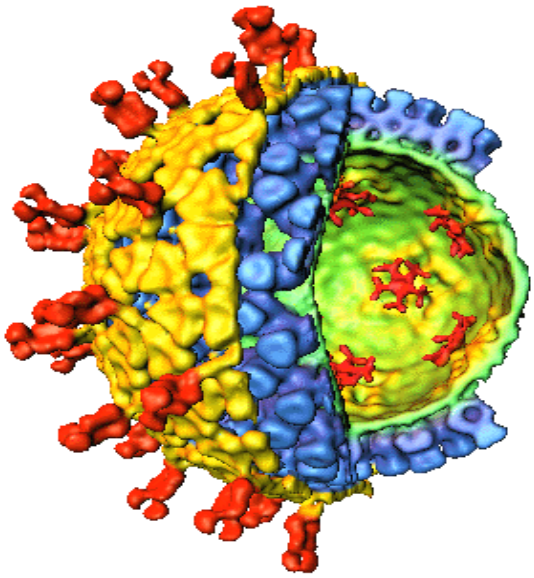

Figure 1.1 Schematic model of Blue Tongue Virus, a close relative in both shape and structure to African Horse Sickness Virus. Note the structural outer capsid (yellow) and inner capsid (blue).

1.3.2 Enzootic and epizootic range

The range of organisms and viruses is defined in terms of natural, long term occurrence and temporary departures from this region. The region where an organism occurs naturally and persistently is known as its endemic range. In the case of viruses, this is known as its enzootic range. Any excursions from its enzootic range are known as epizootics. AHSV is enzootic to sub-Saharan Africa and possibly Yemen in the Arabian Peninsula (Mellor and Hamblin, 2004). In South Africa, it is locally enzootic to the lowveld and bushveld of the Northern Transvaal, KwaZulu Natal and the Eastern Cape Province, where the most common serotypes of the virus, in order of prevalence, are serotypes 2, 4, 1, 6, 7 and 9 (Bremer *et al*., 2000). It does however often exceed its regular boundaries and epizootics can occur in the high lying areas of South Africa. In 1998, about 100 horses died in the Clarens Valley, an area of high elevation and low temperatures (Meiswinkel & Paweska, 2003). Epizootics also occur outside of sub-Saharan Africa. In 1959 - 1961, an enormous epizootic of AHSV serotype 9 occurred in the Arabian peninsula, the Middle East, Afganistan, Pakistan and India where 300 000 horses were lost (Mellor, 1993). Similar epizootics have occurred in Morocco (1989 -1991), and the Iberian Peninsula (1987 – 1990) (Mellor, 1993). Until recently, no epizootic has

lasted outside of southern Africa for more than 2-3 consecutive years. But with new epizootics in Spain and North Africa spanning 5 or more years, a new pattern may be occurring caused by modifications of the climate in these areas (Mellor, 1993).

Epizootics occur if infected animals are transported via air, land or sea to an area where a suitable vector resides. In North America, the midge *Culicoides variipennis sonorensis* has been shown to be a highly effective vector of African horse sickness virus (Welby *et al.,* 1996). It is for this reason that a very tight control is placed on the movement of horses and horse products from areas where AHS is endemic. AHS has achieved O.I.E. (Office International des Epizooties) List A status and is a notifiable disease (O.I.E. ,2006). For this reason, any animals arriving to or leaving South Africa must undergo a quarantine period to confirm their disease status. For this reason, Meiswinkel (1997) proposed an ideal zone along the sandy dunes of the Eastern Cape province that could be used for such a quarantine zone. A possible site was assessed by Rawlings *et al*. (2003) based on an enormous two year collection at 39 sites across South Africa. One site was potentially identified but it was bordered by two sites of high vector populations and so was ruled out. They concluded that none of the 8 clusters analysed were suitable for another AHS free quarantine zone. At present, an area in Cape Town in the Western Cape Province (Figure 1.2) is being used as an AHS controlled area (O.B.P., 2004).

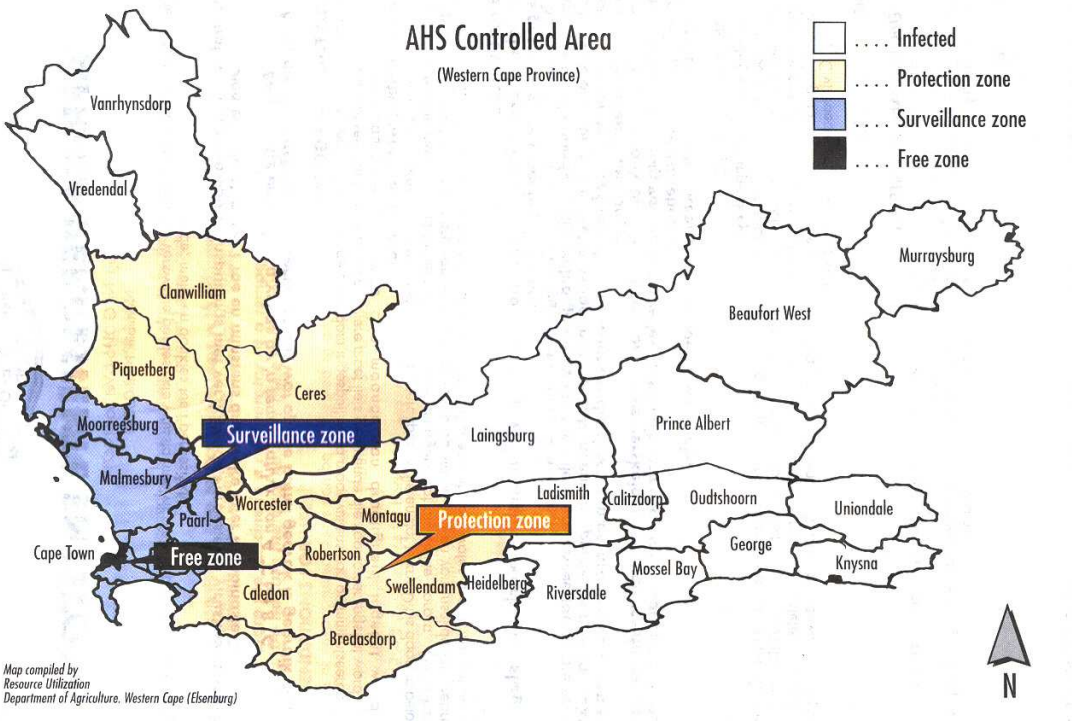

Figure 1.2 South African AHSV free quarantine zone and surrounding buffer zone in the Western Cape province (O.B.P., 2004).

1.4 Ceratopogonidae

1.4.1 Introduction

Midges have many common names throughout the world and are known for their tiny size (Figure 1.3) and painfully itchy bites. Known most commonly as *no-see-ums* or *punkies* in the USA, they are also known as *brulôt* (French Canada), *5 O's* (southern USA states), *moose flies* (Alaska), *jejenes* (Latin America), *maruins* (Brazil), *kuiki* (India), *nukaka* (Japan), *merotoe* (Sumatra) and *no-no's* (Polynesia) (Mullen, 2002). Midges are insects in the order Diptera (flies) and fall into the family Ceratopogonidae (ser-ATO-pog-o-nid-ee). Within this family, there are 125 genera with about 5500 species (Mellor *et al.,* 2000). Of these genera only 4 are known to attack humans and animals (Mullen, 2002). They are *Leptoconops, Lasiohelea, Culicoides*, and *Austroconops macmillani* (the only known species in its Australian genus). Within each genus there is a large variety in species numbers with *Culicoides* having by far the most diverse with 1400 species (Mellor *et al.,* 2000). *Leptoconops* has about 80 species and *Lasiohelea* with about 50 (Mullen, 2002). It

is the *Culicoides* genus which is the most important both from a medical and veterinary stand point.

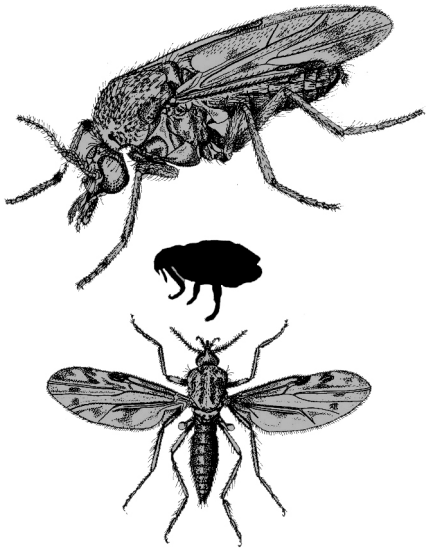

Figure 1.3 Side and top view of a *Culicoides* spp. midge with size comparison to a flea (centre) (Scholtz & Holm, 1985).

Culicoides are found worldwide in a huge range of eco-zones. Venter *et al.* (1996) gave a broad description of habitat ranges as; dry (169mm/annum) to wet areas (927mm/annum), summer or winter rainfall, between 100m-2800 m altitude (although Service (1971) and Mullen (2002) have both found *Culicoides* species at sea level). They have also been found in both cold climates (with yearly temperatures ranging from 4.1ºC to 19.6ºC) and hot climates (with a yearly range from 17.3ºC to 29.4ºC) (Venter *et al* .,1996).

Originally classified under the Chironomidae due to their small size and heamatophagous habit (Mönnig, 1941), their taxonomic status has since been revised and they have been shown by Saether (2000) to be a taxonomically distinct family.

1.4.2 *Culicoides*

1.4.2.1 Introduction

The species within the *Culicoides* genus are amongst the smallest haematophagous (blood sucking) flies, with an adult body length of only 1-2.5mm. (Mellor, 2000; Musuka *et al*., 2001;; Mullen, 2002) . That is many times smaller than the size of a match head (Figure 1.4). 96% of the species within the genus are obligate bloodsuckers (Meiswinkel *et al*., 1994), meaning that the females need to obtain a blood meal in order to develop a batch of eggs (Wittman & Baylis, 2000).

Figure 1.4 Comparative sizes of *Culicoides* species and a match head (Author´s own photograph).

They bite all manner of animals with their piercing mouthparts and females that have just engorged can be noted by the pigmentation in their abdomens (Figure 1.5). Some species can be extremely populous with a 1000 fold increase in numbers occurring when climatic conditions are correct (Mellor *et al*., 1975; cited in Mellor & Hamblin, 2004). In Scotland, *Culicoides impunctis* females are a major

pest of livestock and farm workers and negatively impact on the local economy by their biting attacks on man, driving forestry workers away with their dense choking clouds, and by making tourism impossible because of their painful bites. Their attacks also drive red deer off rich, low-lying pastures and up into poor-nutrient high slopes (Mands *et al*., 2004)

Figure 1.5 An engorged female *Culicoides* spp. showing pigmentation of the abdomen with blood (Author´s own photograph).

1.4.2.2 Distribution

The *Culicoides imicola* species complex is comprised of 10 very closely related species and is of huge importance as a disease vector (Mellor *et al*., 2000). Of the 10 species comprising this complex in South Africa, seven are confirmed (*C. imicola sensu stricto; C. brevitarsis* Keiffer; *C. pseudopallidipennis* Clastrier; *C. nudipalpis* Delfinado; *C. bolitinos* Meiswinkel; *C. miombo* Meiswinkel; *C. loxodontis* Meiswinkel) and 3 are provisional (*C. tutti-frutti* Meiswinkel; *C. kwagga* Meiswinkel; and *C.sp. #103* Meiswinkel)(Linton *et al.,* 2002; Dallas *et al*., 2003).

The properly named *Culicoides imicola* Keiffer (cited as *Culicoides pallidipennis* Carter, Ingram & McFie) is an Afro-Asiatic insect that prefers warm climates (Lubega & Khamala 1976; Mellor, 1993).

The only gross morphological difference between *Culicoides* species that can be easily and visibly noted is the patternation on the wings (Figure 1.11). *Culicoides*

midges are found across Africa in the sub-Saharan countries of South Africa, Zimbabwe, and Kenya as well as Morocco, Sudan and Egypt (Bouayoune et al 1998). They are common across Europe and the Middle East and have been collected as far north as 41º17'N in Portugal (Venter *et al.,* 1996; Rawlings *et al*.,1998 (abst,); Mellor *et al.,* 2000; , Wittman & Baylis, 2000). They are more abundant in warm, low lying areas than in areas with cold winters that experience a lot of frost, and are less abundant in warm areas that are very dry or in areas with winter rainfall, dry summers and cold winters (Venter *et al*.,1996). In a study done in Spain, *C. imicola* was found at 26 of the 27 sites, in warm low lying areas (Rawlings *et al.,* 1998). *C. imicola* was also indicated as the most likely vector of AHS and Blue tongue by Ortega *et al*. (1998).

1.4.2.3 Habitat distribution

Culicoides breed in a wide variety of habitats. They are neither terrestrial nor aquatic but occupy an in-between ecotone where there is a wet substrate rather than open water (Mellor *et al.,* 2000). They have been found to breed in almost any available substrate. They breed in both salt and fresh water marshes, animal dung, rotting vegetation such as fallen banana trees and forest floor cover, in tree holes, in waterlogged pastures, on beaches, around leaking irrigation pipes and water troughs, in muddy farm yards, and in areas of both high and low organic and faecal matter content. They have been found in sewage installations and drainage channels. Most commonly they are found along the edges of pools, streams and any permanent water body (Service 1971; Braverman *et al.,* 1974; Lubega & Khamala, 1976; Mellor & Pitzoltis, 1979; Edwards, 1982; Mellor *et al.,* 2000; Mullen, 2002).

1.4.2.4 Life cycle and ecology

The life cycle of *Culicoides* is a simple holometabolous one. That is, a life cycle where a pupal stage is interposed between the larval and the adult form (I.N.R.A., 2006). The eggs are laid in batches which adhere to the substrate and are approximately 400µm long and 50µm wide. The eggs are not resistant to drying out

and must therefore be laid in an area which will not desiccate for at least 7 days after laying (Mellor *et al.,* 2000). Eggs develop for between 2 and 7 days and then hatch into thin, elongate larvae that move in a serpentine manner through the substrate (Figure 1.6).

Figure 1.6 *Culicoides* larva (E.L., 2006).

The larvae eat nematodes (Edwards, 1982), particles of vegetable matter, rotifers, protozoa and other small arthropods. They do not have opposable mandibles but rather have a pair of pincer like mandibles that tear and scrape at food (Mullen, 2002) There are four larval instars with the larva moulting and growing larger each time. The larval stage can last between four days and several weeks. (Mellor *et al.,* 2000). The onset of winter and the first frost arrests the development and pupation of many species thus causing them to fall into a state of diapause in either the fourth larval instar or the pupal stage (Mellor *et al.,* 2000; Mullen, 2002). Thus the larval stage can be extended to up to a year in some species with about 8 months being spend over-wintering (Mullen, 2002). In a study done on *Culicoides subimmaculatus* in Australia, the developmental times were given as the number of day-degrees taken per life stage. 91 day degrees over the 9.7ºC zero development point was needed to complete egg development. 591 day degrees over the 9.5ºC zero development point to complete larval development and 38 day degrees over the 14.1 ºC zero development point to complete pupal development (Edwards, 1982). Thus at 25ºC, a total life cycle would take 47.6 days from the egg batch of one generation to the next. If temperatures were to consistently drop below the lowest zero development point of 9.5ºC, then diapause would occur and the insect would not develop until temperatures rose again. It is this mechanism that is seen to occur with our own South African species, *Culicoides imicola,* which is seen to

abound during the hot months but then almost disappear during the cold winters (Meiswinkel *et al.,* 1994; Venter *et al.,* 1996). This phenomenon is demonstrated well in the readings taken at Onderstepoort from 1963 to 1970 (Figure 1.7).

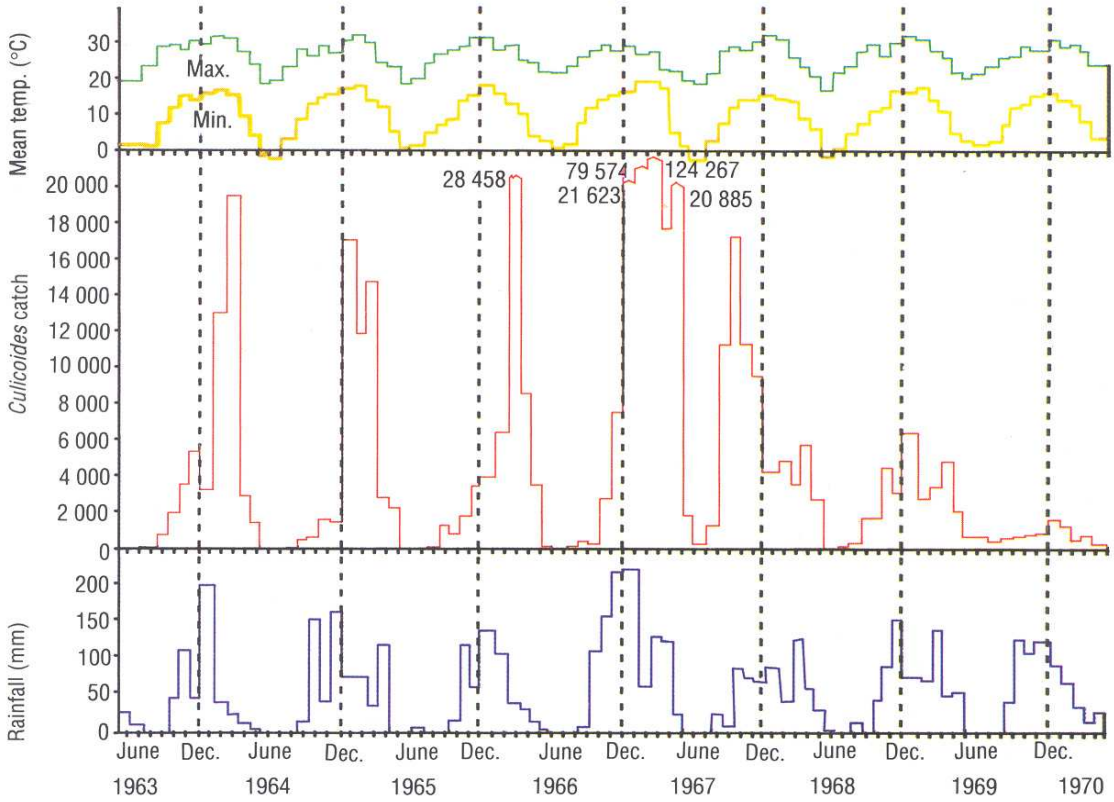

Figure 1.7 Numbers of *Culicoides* species caught at Onderstepoort in relation to rainfall and temperature (Coëtzer & Erasmus, 1994).

The pupae are small, and are loosely attached to the substrate. They take between two days to three weeks to develop during which time they have limited mobility (Mellor *et al.,* 2000). After this time, the adult emerges as an imago and finally matures into an adult midge. Female *C. subimmaculatus* were shown by Edwards (1982) to develop slower than the males by about two days. Across the genus, there are many different life cycles. Some are univoltine, having only one generation per year. Others are bivoltine causing peaks in numbers twice a year and still others are multivoltine thus leading to elevated population numbers throughout the year (Mullen, 2002).

Most species of *Culicoides* females need to feed on blood to get the necessary energy and protein required to mature a batch of eggs (Lord *et al.,* 1996). Many

species however are autogenous (C*. barbosai* and *C. furens,)* carrying fat reserves over from the larval stage and thus being able to lay the first batch of eggs without requiring the energy from a blood meal to do so (Linley, 1966; Mullen, 2002). Linley (1966) found that female *C. barbosai* could not survive long enough on just one blood meal after they had laid their first batch of eggs. The females were offered extra carbohydrate in the form of honey-soaked cotton wool and, using this method, he was able to maintain the females through numerous egg batches. This indicates that, while it is commonly known that the males feed only on nectar, the females might too need extra energy to sustain them. The activity of adult midges has been shown to be crepuscular, that is, they exhibit peaks in activity around the hours of sun rise and sunset (Holbrook & Bobian, 1989) but further research done on *C. imicola* in Kenya by Walker (1977) states that this is not always the case in the tropics. The two most important species in South Africa, *C. imicola* and *C. bolitinos,* are crepuscular (Meiswinkel *et al.,* 2000) and their activity levels are the highest in warm, humid and calm periods (Boorman, 1993). Adult *Culicoides* are not good fliers and their activity is negatively impacted by winds of over 3m/s. Because of their weak flight, the adults only ever disperse a few hundred meters from their breeding sites (Kettle, 1984) and occasionally up to 2-3 km from their breeding sites (Lillie and Jones (1981) cited in Wittman & Baylis, 2000). They have been shown to disperse over huge distances as aerial plankton and can travel up to 200 km in windy conditions (Murray, 1991).

1.4.2.5 Host location and semiochemicals

To locate their hosts, the females use CO_2 and other semiochemical cues. Within these semiochemical compounds are pheromones, kairomones and apneumones (Mordue & Luntz, 2003). Pheromones are typically used in mate location but to date no pheromones have been found to be associated with *Culicoides* midges. Kairomones are compounds used specifically to locate and identify mammalian hosts. Host kairomones are lactic acid, acetone, alkyl phenols, butanone and 1-octen-3-ol (Ritchie *et al.,* 1994; Mordue Luntz, 2003)). Finally apneumones are used to detect oviposition sites. No work has so far been done on apneumones of *Culicoides* midges but the mosquito *Culex quinquefasciatus* has been shown to be strongly attracted to an apneumones when searching for sites in which to lay its eggs (Mordue & Luntz, 2003). In nature, the CO_2 breathed out by animal forms a

downwind plume (Takken & Kline, 1989) which is enhanced by the production of the abovementioned kairomones expelled from the skin and body. When an insect crosses this plume, an “upwind orientation” occurs and the insect follows the plume back to the animal (Kline *et al*.,1994). Once close to the animal, the midges use visual cues to determine the species and site of attack. Control methods utilizing both of these compounds as well as UV light are available on the market. These will be discussed later.

1.5 Vector Borne Diseases

Diseases vectored by *Culicoides* have been well documented. Meiswinkel *et al*. (1994) gave a thorough description of *Culicoides* vectored diseases. They can be nematodes, protozoa or viruses and symptoms can even be in the form of allergic reactions, for example allergic dermatitis (Queensland-itch or sweet-itch). 18 species of filarial nematodes have been isolated from *Culicoides* species as well as 22 different protozoa of both mammals and birds.. Despite all of these, it is the viruses that are transmitted by *Culicoides* species that are predominantly of veterinary and medical interest.

Culicoides species vector a total of 50 arboviruses worldwide. All 50 species fall into 3 families. They are the Bunyaviridae (20 species), Reoviridae (19 species), and the Rhabdoviridae (11 species) (Mellor *et al.,* 2000). Bunyavirid viruses such as Akabane and Shamonda have been isolated in South Africa and Israel from *C. imicola* (Stram *et al*., 2004) and from a host of African wildlife including elephant, blue wildebeest, and the African buffalo. These viruses cause congenital abnormalities in domestic ruminants and poultry. The virus causing bovine ephemeral fever falls into the family Rhabdoviridae. This has been isolated in *C. brevitarsis* in Australia. As *C. bolitinos* is the African sister species to *C. brevitarsis* (Meiswinkel *et al.,* 1994), we can assume that bovine ephemeral fever is vectored the same way naturally in South Africa. Both of the midges develop in the dung of cattle with *C. bolitinos* utilizing the dung of the African buffalo and the blue wildebeest.

The Rhabdoviridae are of great interest in South Africa. Falling into the Rhabdoviridae are the Nyabira virus, Gweru virus, epizootic haemorrhagic disease viruses of deer, bluetongue virus (BTV), African horse sickness virus (AHSV) and equine encephalosis virus (EEV). The Nyabira and Gweru viruses have been related to abortions in sheep, cattle and goats. The epizootic haemorrhagic fever virus is also vectored by a *Culicoides* species. BTV and AHSV are long standing stock-associated viruses in South Africa and are both most commonly vectored by *Culicoides imicola* (Meiswinkel *et al.,* 1994; Venter *et al*., 1996 &, 2000). African horse sickness virus antibodies have also been insolated from elephant and various carnivores near Onderstepoort in north eastern South Africa (Bremer *et al.,* 2000).

1.6 *C. imicola* and *C. bolitinos*

Two species have been proven to be highly competent vectors. These are *Culicoides imicola* and *Culicoides bolitinos* (Du Toit, 1944; Paweska *et al*., 2003; Venter *et al.,* 2000). These two were separated as distinct species by Linton *et al.* (2002) and Dallas *et al.* (2003). *C. imicola* is found world wide and is by far the most important veterinary *Culicoides* species. Both *C. imicola* and *C. bolitinos* are very closely related taxonomically and fall into the *C. imicola species* complex made up of 10 *Culicoides* species (Meiswinkel, 1989). *C. bolitinos* is less important but has been strongly implicated in the transmission of AHS by many workers (Venter *et al.,* 1996; Meiswinkel *et al.,* 2000; Meiswinkel & Paweska 2003). It is the ecologically equivalent sister species to *C. brevitarsis* in Australia (Meiswinkel, 1989). A lot of work has also been done on C*. brevitarsis*; the results of which can be broadly related to our own South African species. The ecologies of *C. imicola* and *C. bolitinos* differ greatly and are shown in Table 1.1.

Table 1.1 Differences in the ecologies of two South African *Culicoides* species; *Culicoides imicola* and *Culicoides bolitinos* (Meiswinkel & Paweska, 2003).

Culicoides imicola	***Culicoides bolitinos***
Immature stages Reside in moist but not waterlogged soils; pupa is unable to float at the water surface; Predominates in moisture retentive, organically enriched, clayey soils which can be unvegetated or covered with short grass (as in grazed pastures) Upper layer of soils must remain moist for ≥7 days to sustain larvae through four moults; this restricts the species at almost any altitude to more level areas that are slower draining. Absent from quick draining, nutrient poor, sandy, coastal soils. Only small populations occur in sloping terrain here soils desiccate rapidly due to water runoff. During episodic periods of above average precipitation, *C. imicola* populations increase as moist soils become more widely available. Restricted to 300-700 mm rainfall isohyets as soils become leached of nutrients at higher precipitation levels. Adults Most-widespread and most-abundant *Culicoides* species in South Africa; feeds on a wide variety of domesticated livestock. Shows large variation in abundance; 200-fold increase in years of above average rainfall. Largest single light trap collection recorded 1 000 000. Widespread in sub-Saharan Africa, and on offshore islands; also occurs in North Africa, and up to the 43rd parallel in European countries bordering the Mediterranean; occurs also in the Near, Middle and Far East up to southern China and Vietnam.	Immature stages Resides exclusively in the dung of cattle, the African buffalo and the blue wildebeest; will occur in any area where the above herbivores are found. . Occurs on both flat and sloping terrain. Following deposition of eggs in fresh dung, larvae require 7-10 days to reach maturity. The coprophilic larvae develop independently of soil moisture and quality; highest abundance achieved in areas where cattle are maintained on more sandy soils. Average annual precipitation does not affect its geographical distribution Adults Almost as widespread as *C. imicola* but , on average, is one order-of-magnitude less abundant; rarely found in co-dominance with *C. imicola* Wide choice of hosts Adult abundance variable but proportional to the amount of dung available- this in turn dictated by animal biomass per unit area. Largest single light trap collection recorded 13 000. Currently understood to be restricted to sub-Saharan Africa; recorded from only 8 continental countries (South Africa, Lesotho, Botswana, Zimbabwe, Malawi, Kenya, Nigeria, and the Ivory Coast), and from the islands of Madagascar and Mauritius.

There have been many species of *Culicoides* implicated in the spread of African horse sickness. Paweska *et al*. (2003) showed from laboratory fed midges that eight midge species are orally susceptible to the various serotypes of the virus. Table 1.2 shows the species and the serotype that were recovered after a ten day extrinsic incubation period.

Table 1.2 Oral susceptibility to AHSV serotypes amongst South African *Culicoides species* (after Paweska *et al., 2003).*

Species name	**AHSV serotype recovered**
C. imicola Keiffer	2 & 7
C. bolitinos Meiswinkel	2 & 3
C. engubandei de Meillon	4
C. magnus Colaço	3 & 4
C. zuluensis de Meillon	2 & 4
C. pycnostictus Ingram & Macfie	2
C. bedfordi Ingram & Macfie	7
C. dutoiti de Meillon	7

*C. imicola (*Figure 1.8) is the only *Culicoides* species capable of a 200 fold increase in numbers seasonally (Meiswinkel & Paweska, 2003) and can reach astounding numbers. In a single light trap in one night at Onderstepoort, Meiswinkel (1998) caught one million individuals with *C.imicola* commonly comprising >99% of the total *Culicoides* fauna caught. It is commonly associated with livestock and was found by Barnard (1997) to be attracted to paddocks in which cows, sheep and horses were kept. It is a species that prefers to remain out side stables and buildings and will readily attack any animal that is left outside during its crepuscular hours of activity (Barnard 1997; Meiswinkel *et al*., 2000). It was found to be active throughout the night in Kenya by Walker (1977) and thus is even more of a threat to local animals. It breeds in moist soil and has a varied range of ecotopes in which it is willing to lay eggs (Randall, 1982). It has been found to lay its eggs in moist soil exclusively by Walker (1977) and, in Kenya, Lubega & Khamala (1976) found it breeding both in moist soil and in cattle dung. A similar situation was also observed by Nevill (1968). It is the sibling species to *C. miombo* from the Gambia (Rawlings *et al.,* 1998).

Figure 1.8 Female and male *Culicoides imicola.* Note the large body of the female and the feathery antennae of the male that are used to receive pheromonal stimulate from the female.

C. bolitinos is less populous than *C. imicola* and is generally of an order-of-magnitude less abundant. It is coprophilic, meaning that it breeds exclusively in dung. To date, the only three herbivores that it has been associated with are the African buffalo (*Synceros caffer*), the blue wildebeest (*Connochaetes taurinus*), and domestic cattle (*Bos spp.)* (Meiswinkel & Paweska, 2000). It has never been found to breed in the dung of horses (*Equus caballus*) (Meiswinkel *et al.,* 2000). It is never found in co-dominance with *C. imicola* and, because it breeds exclusively in dung, is not restricted to certain soil types for larval development (Meiswinkel, 1997). It can also tolerate far colder climates than *C. imicola* (Venter *et al.,* 1996*).* In the Eastern Cape it was found to be highly dominant and numerous, thus jeopardizing plans for an AHS free zone in South Africa that could be used for a quarantine zone (Meiswinkel, 1997). It was shown by Meiswinkel & Paweska (2003) that *C. bolitinos* is a vector for African horse sickness virus. Venter *et al.* (2000) proved the oral susceptibility of *C. bolitinos* to AHSV serotypes 3, 5, and 8.

This was evidenced in 1998 when roughly 100 horses died of the disease in 90 days in the Clarens Valley, a high, cool mountainous region. *C. imicola* comprised less than one percent of the total catch and was only found at one third of the farms sampled while *C. bolitinos* comprised 65% of the total catch and was found at all the

farms sampled. This and other ecological information caused Venter *et al.,* (1996) to give *C. bolitinos* a vector rating of 29.7%.

1.6.1 Climatic effects on *Culicoides* species

Culicoides species occur all over Africa and their numbers are largely dependant on climatic variables. Across the region, each population has very different cycles. In attempting to find commonality within the group, Mellor *et al.* (2000) reviewed the seasonality of the *Culicoides* species in Africa and found a huge variation (Table 1.3). A pattern was found by Baylis *et al.* (1999, cited in Mellor & Hamblin, 2004) for the epidemics of AHS is South Africa. Twelve of the last thirteen epidemics have occurred after heavy rains following a period of drought. He showed that the epidemics coincided with the El Nino Southern Oscillation (ENSO) fluctuations in South Africa. Barnard (1998) showed that all major outbreaks of AHS were associated with periods of very high precipitation.

Table 1.3 Seasonality of *Culicoides imicola* populations in Africa (Bouayoune *et al.,* 1998).

Country	**Seasonality**
Morocco Israel	Populations peak at the end of the summer months after the last rains.
South Africa winter rainfall areas	Adults are absent during winter when the highest precipitation occurs.
Nigeria	Populations peak just after the rainy season at the coldest part of the year. Very low numbers at the hottest driest part of the year.
Sudan South Africa summer rainfall areas	Populations peak at the end of the rainy seasons towards the end of summer.

The effects of climatic variables on *Culicoides* midges are well known. Wind speed has been found to negatively affect activity of midges. Winds of 3m/s and 2.2 m/s inhibit the flight activity of *C. imicola* and *C. bolitinos* respectively (Walker, 1977; Whitman & Baylis 2000) and adult survival has also been reported as being

negatively correlated with wind speed. Murray (1991) found midges being blown 100-200 km inland from their breeding sites on the coast of Australia. He used these findings to explain how an epizootic may occur even when the species of vector is not common in the area. After an outbreak of Japanese encephalosis in northern Australia, Johansen *et al.* (2003) did a study of wind blown insects and found that midges can get blown across the sea from Papua New Guinea to the Torres Strait of Australia on winds of just 5-15 km/hr. These insects were caught in an aerial trap that was suspended 70 – 150 meters high, showing that these small insects are highly prone to wind dispersal. How the midges get to an area has been shown by Lord *et al.* (1997) not to matter in respect of the time of vaccination, because the lag time between vaccination and protection is longer than the latent period of the infection in the hosts. So even if a vector were to be blown into an area the chances would still be high that the susceptible horses would contract African horse sickness before a regional alert could be given,

Temperature has long been known to affect the activity of *Culicoides* species. It was found that flight is inhibited at temperatures below 10℃ for *C. varipennis* and 18℃ for *C. brevitarsis* (Whitman & Baylis, 2000). In a study of various midge species in Scotland, a negative correlation with temperature was found, except in the case of the activity of female *C. impunctis* midges (Blackwell, 1997). In Kenya, Walker (1977) found a positive correlation between the activity of *C. imicola* (cited as *C. pallidipennis)* and temperature. In samples taken over a three year period across the entire land mass of Portugal, *C. imicola* was only present during winter months at a density of one percent of that of the summer months (Capela *et al.,* 2003). Kettle *et al.* (1998) also found a positive correlation to temperature in the activity of only female *C. brevitarsis* but not the males of the species.

Bishop *et al.* (1996) found that when *C. brevitarsis* larvae were reared under different temperatures, more males were produced at low temperatures and high rearing temperatures produced more females. They found that larval development stops at 17℃. If larvae were kept at 17℃ for a p eriod of time and then returned to their optimal 28℃, fewer adults emerged the longer they were left in the cold temperatures. Adults reared at just 12℃ were about one third the size of adults reared under optimal conditions. Lindley & Hinds (1976; cited in Whitman & Baylis,

2000) also found that more, less fecund females were produced when larvae were reared in warm temperatures as opposed to cold ones. For *C. brevitarsis* in Australia, the upper lethal limit for adults was 43℃ (Bishop *et al.,* 1996).

Relative Humidity has also been linked to midge activity. *C. impunctis* females are more active at higher relative humidities as are both sexes of *C. imicola* (Walker 1977; Blackwell,1997,). At the coast, Kettle *et al.* (1998) found a correlation between the tidal cycle and their catches of male *C. brevitarsis* but found that activity was not affected by either lunar cycles or tidal maximums. Midge activity stops in heavy rain as they do not fly during downpours (Murray, 1991).

Light intensity is thought to be the trigger for the crepuscular nature of many midges (Holbrook & Bobian, 1989). This has been used in many collectors to maximize catch efficiency (Holbrook & Bobian, 1989; Blackwell, 1997; Kettle *et al*., 1998; Murray, 1991; Bishop *et al.,* 2000; Johansen *et al*., 2003; Meiswinkel & Paweska, 2003; Rawlings *et al*., 2003). While trap catches were lowest at full moon, no correlation could be made with midge activity by Bishop *et al*. (2000) because they could not correlate the affects of the moon to the activity of midges but rather to the effectiveness of the trap that they used.

Apart from climatic factors, many other factors have been tested to see their effects on midge activity. These have mostly been done via the comparisons of land-gathered data and images taken via satellite. This have included soil data, humidity and temperature data, windspeed and the normalised difference vegetation index which is a measure of the plant activity in an area. These models will be dealt with separately.

The main point regarding the relationship between disease vectors and climate is that while the climate at present allows for large populations of midges to be present, a changing climate might further increase possible breeding sites and therefore cause booms in numbers of disease vectors worldwide. As global warming increasingly heats the globe, species limits will be pushed farther and farther north into areas where it was previously too cold for them to inhabit.

1.6.2 Virogenesis

Much work has been done on the effects of climatic conditions on both the vector species and the rate at which the virus is generated in them (Welby *et al.,* 1996; Mellor *et al.* 2000; Wittman *et al.* 2002; Capela *et al.* 2003). The general conclusions of this work were that as temperatures rose to around 25℃, the rate of viral transmission in the field rose significantly. Welby *et al.* (1996) discovered that maximum rate of virogenesis occurred at 25℃ and dropped steadily as temperatures dropped. It stopped altogether at 10°C. This zero replication point was found to be at 15℃ by Mellor *et al.* (2000). Wittman *et al.* (2002) found that the rate at which AHSV serotype 4 is incubated in *C. sonorensis* was reduced from 18 days at 15ºC to only 5.7 days at 30ºC. This could be one of the overwintering mechanisms in place for this species. It was also found by Capela *et al.* (2003) that AHSV does not occur above the 44℃N latitude line. They suggested that it is the low average annual temperature that stops effective virogenesis at these latitudes. Adult longevity was shown to be negatively correlated to temperature with longevity dropping three fold when temperatures rose from 15°C to 30℃ (Mellor *et al.,* 2000; Whitman, 2000). This would suggest that high temperatures are not good for viral transmission but, egg development rates within the females increase as temperature rises and so there is a shorter period between batches of eggs and so more batches are laid and thus more blood meals required by the vector females (Lindley 1966; Wittman & Baylis, 2000). This increases the midge's vector potential (Whitman *et al.,* 2002).

1.6.3 Overwintering

Populations of *Culicoides imicola* midges are seasonal and it is this phenomenon that poses the hardest question. How does the virus overwinter? That is, how can the virus stay active during a period of cold temperatures that is not conducive to its transmission? The virus can not replicate below 15℃ (Mellor & Hamblin, 2004) and so must find a safe place to stay dormant during the entire winter. There are only a few places that it can remain active in host animals; in the insect vector and in the mammal host.

In the insect vector, it can either remain active in the adults or be passed on to the next generation by the females via the eggs she lays. The insect vector only lives a few weeks, usually 10-20 days but up to 90 days in exceptional cases (Mellor *et al*., 2000). Nevill (1967) showed that viability of eggs dropped after they had been kept at 6.5℃ for more than 7 days and thus ruled out the chances of insect surviving the winter in the egg stage. Studies reported on in Whitman & Baylis (2000) also showed that there is no transovarial transmission of the virus in *Culicoides* species. It has since been found that most *Culicoides* species can survive for long periods as the fourth instar larvae (Whitman & Baylis, 2000, Mullen, 2002).These two conclusions prove that the virus can not remain active in the insect because firstly the adults do not live through the four or five winter months, and secondly the overwintering larvae are not infected with the virus until they emerge as adults and get infected by biting a mammal host. It has also been shown that the proportion of the vector population that is infected with the virus is extremely small. Only 1 in 35 000 females tested by Muller *et al.* (1982) in Whitman & Baylis (2000) were capable of vectoring the virus. This would mean that the virus could be vectored in the midge in the summer when population numbers are huge but it is highly unlikely that vectoring occurs in winter when population numbers crash so dramatically.

This only leaves the mammalian host as a possible reservoir in which the virus can remain active. Dogs were thought to be a reservoir but in a test done in Israel by Braverman & Chizov-Ginzburg (1996), none of the 1123 test dogs were actually bitten by the *Culicoides* midges. They reported that the confirmed cases of AHS in dogs arose from dogs consuming infected meat. Horses either recover from the disease or die from it. The dead horses are removed from the possible population and so do not enter the dynamics of the disease any further (Lord *et al.,* 1996). Only the sick and surviving animals are viraemic (capable of passing on the disease to a vector). Horses are only viraemic for 4-8 days and never more than 21 days (Coëtzer & Erasmus, 1994). In donkeys the levels of virus in the blood are only at very low titres ($<1.5X10^2$ $\log_{10}$ $TCID_{50/ml}$) and they too only exhibit a short 4-week viraemia (Coëtzer & Erasmus, 1994; Barnard, 1998). In the zebra of Kruger National Park in north eastern South Africa, antibodies to all nine of the AHSV serotypes were found and, although the zebra stayed viraemic for 6-7 weeks, little evidence supported that they were definitely the hosts in which the virus

overwintered (Mellor & Hamblin, 2004). But in a study of the aetiology of the virus and the epizootics, Mellor & Hamblin (2004) suggested that it is the presence of zebra that helps the virus continue as no epizootic has been able to last outside of sub Saharan Africa with no zebra. Hamblin *et al.* (1998) showed that viraemia in donkeys only lasted about twelve days and at very low levels and stated that they did not consider donkeys to be a long term reservoir of the disease. Barnard (1998) suggested that if the zebra population was large enough to allow cycling of the virus among the individuals, then it would be possible to get a focal point around which a new spread of the disease could center. An interesting suggestion was made concerning the *C. imicola* midge populations in Portugal. During the winter, *C. imicola* numbers drop drastically but populations of *C. pulcaris and C. obseletus* remain very high. Capela *et al.* (2003) postulated that interspecific virus cycling keeps AHSV active from one summer to the next. When it is too cold for large numbers of *C. imicola* to be present, the other two species transmit the virus at a high enough level to maintain it activity. This type of interspecific cycling is shown in Figure 1.9.

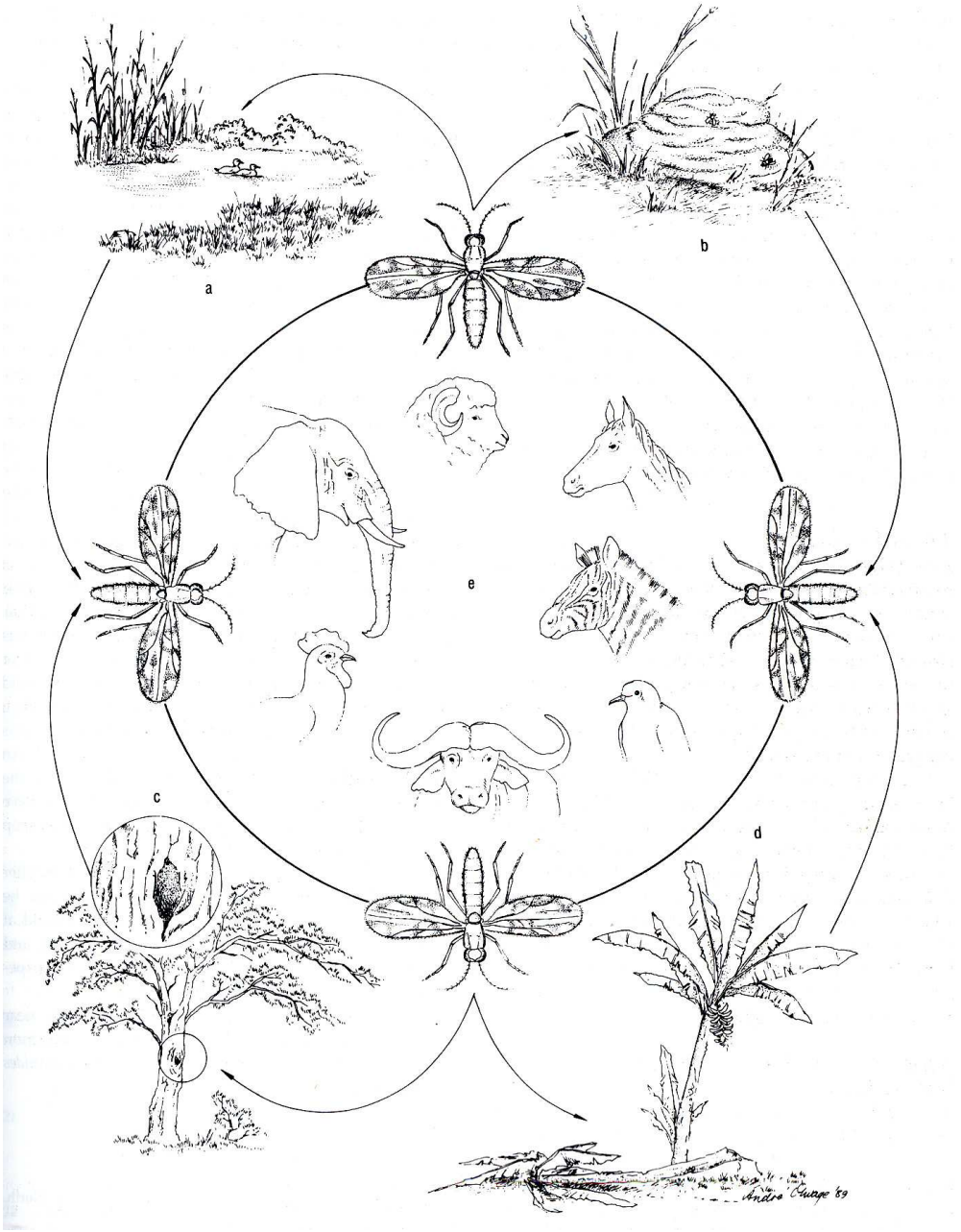

Figure 1.9 Interspecific virus cycling. Note the variety of hosts, vectors and larval habitats that combine to make control very difficult (Hubers, 2006).

In South Africa it is common knowledge that the first frost generally stops the disease. Table 1.4 shows the monthly infections for the 2005/2006 outbreak of the disease. The first frost is generally in June and it can be seen that all incidences of AHS stopped in May of 2006. In a seven year study by Nevill (1971; cited in Mellor *et al.,* 2004), the only year to yield midges catches throughout the year coincided

with the warmest winter with little or no frost. In northeast South Africa, AHS occurs year round because the climate is mild and the area does not experience heavy frosts and so the vectors do not die out each winter (Barnard, 1998).

Table 1.4 Statistics from the 2005/2006 AHS outbreak in South Africa. (AHS website, 2006).

Duration (months)	7
Total confirmed cases	844
Died	148
Survived	696
Confirmed Cases by Month	
Nov-05	1
Dec-05	0
Jan-06	18
Feb-06	80
Mar-06	622
Apr-06	105
May-06	18

1.7 GeoSat Modeling of Vector Populations.

Many studies have been done in a predictive capacity for *Culicoides* populations both in Europe and in Africa. These all try to predict, via correlation of satellite imagery and land measured variables, when and where the next large population of disease vectors is likely to occur. There are three main reasons for this approach. Firstly, the variables produced by satellite imagery are better correlated to ecologies of whole regions than are locally focused readings (Baylis *et al.,* 1998). Secondly, they have a global coverage and therefore predictions can be made easily from one country to another, and thirdly, because the data is readily available at little or no cost (Baylis *et al.,* 1999). Conte *et al.* (2004) did a Spatial Process Model (SPM) based on the geographic information system for Italy. They considered 7 variables upon which the model would be based. They were; elevation, land use, aridity index, lithologic environment, animal population density, daily minimum temperatures and finally the monthly Normalised Difference Vegetation Index (NDVI) which is a

measure of the activity of chlorophyll in an area and thus a measure of vegetation activity. Their results were inconclusive and suggested a higher weighting be given to one or other of their parameters. Another study done by Baylis *et al.* (1998) in Morocco showed that 50% of the variance noted in the annually-averaged mean-daily catches of *C. imicola* were explained by a model using two variables, the average and mean annual minimum NDVI and wind speed. They found no correlation between population numbers and the following variables that they measured: air temperature, soil temperature, relative humidity, saturation deficit, rainfall, altitude, and maximum NDVI. They suggested that NDVI is an important variable as it is a factor of soil moisture, which also affects ground dwelling insects. Capela *et al.* (2003) found that these models tested on populations in Portugal worked very well for the prediction of *C. imicola* midges.

1.8 Control

1.8.1 Control of Vectors

Many methods have had varying successes at controlling these midge populations. There are four main types of control that can be used against blood sucking insects. They are biological control, physical control, chemical control and trapping. Biological control is the purposeful introduction, by man, of parasites, predators, and/or pathogenic microorganisms to reduce or suppress populations of plant or animal pests (E.L., 2006). Only a very small amount of research has been done on biological control of *Culicoides* species. Bishop *et al.* (2005) found that the dung larval habitat of *C. brevitarsis* can, to a certain extent, be altered by dung beetles *(Onthophagus gazelle* L.) in Australia but the effects of population reduction were not enough to warrant the beetles as suitable biocontrol species. Unkels *et al.* (2004) tested the entomopathogenic fungus (C*ulicinomyces clavisporus)* against *C. nubeculosus* and discovered that it had a considerable larvicidal effect. Further research is therefore needed to determine the species range of the fungus.

Chemical control involves the use of man-made or naturally occurring compounds to kill or deter the growth and development of pest species. Larviciding with oils is used widely against mosquito pests but this can not be adapted to *Culicoides* spp. because they do not live in open water bodies as do mosquitoes. Fogging

vegetation and stable yards with DDT and Dieldrin (a DDT metabolite) was used very effectively in the past to control all manner of insects. Due to their build up in food chains and their consequent effects on bird life they have been banned in recent years in South Africa. Other strategies and compounds have therefore been implemented. The use of malathione, Abate© or Dursban© give good control of midges (Service, 1971) but their effect is only transitory. Abate© was reported by Mellor & Hamblin (2004) to have only a 30 day efficacy. Pyrethrum based chemicals are good for fogging and knocking down adult insects but, because of their rapid degradation, are not useful for larviciding. Ultra low volume sprayers are now implemented to deliver micro droplets of chemical to active adults and thus reduce the bite load on horses. More research needs to be done concerning the correct times and cycles of spraying to get the maximum control. Service (1971) found that in temperate regions, such a Europe, aquatic control of the larvae is most effective but in tropical and sub tropical regions, such as South Africa, adult control is most effective.

Service (1971) suggested physical control of midges where the habitats of larvae are destroyed or altered to make them unsuitable. Draining large areas of wet land has been used in the control of mosquito larvae but this method would be difficult to apply to *Culicoides* midges because they do not tend to all breed in one site but rather in many small pockets throughout the surrounding area (Randall, 1982).

Trapping is a large field of interest and there are many ways in which the concept of removing unwanted insects can be applied. Listed below these include;

Light emitting electrocution traps
Sticky traps
Bait traps
Light emitting suction traps
Out trapping flies to get them away from the target animals

1.8.1.1 Light Emitting Electrocution Traps

These are most commonly used to keep flies away from food preparation areas and are often used indoors. The structure of these traps is very simple. A UV light is suspended between two grids of electrified wire. As insects are attracted to the light, they simultaneously touch two of the wire strands where they complete a short circuit of the current resulting in their combustion. This method is effective for control of large insects such as house flies and moths. There are, however many downsides to these traps. They require installation in an open area of the premises and need to be plugged in all of the time. Only when the insect touches two of the wires, is it electrocuted. This is not a suitable trap for very small insects like midges as they are too small to touch two of the wires simultaneously and so simply fly through the trap. The traps attract all insects that are positively phototactic. This results in many advantageous creatures dying in these traps. Geckoes and lizards are attracted to the insects near these traps and are also often killed. The sound and smell of the burning insects can sometimes be offensive to people.

1.8.1.2 Sticky Traps

Sticky traps are any trap that uses a sticky substance to catch animals. Many people are familiar with fly strips. These are hung from the ceiling of roof of a building and randomly catch any passing insects. Sticky traps can also be in the form of sticky sheets of card that are coated in glue and hung. Once again there are several pitfalls to this type of trap. As there is no attractant involved in these traps, the catch tends to be a very small proportion of the total insect population. Insects are caught at random and there is no discrepancy between the target insects and others. They are messy to set up, unsightly, and often catch airborne debris and dust thereby reducing their stickiness and effectiveness. The fill up quickly and need to be replaced frequently.

1.8.1.3 Bait Traps

Bait traps have the added advantage over sticky traps in that they actively attract insects to the trap. Baits can be very specific to the pest insect and so a degree of

sorting is conferred on the insect population. Using 'red top' fly traps as an example, beneficial insects such as bees, and butterflies are not attracted to the smell of these traps and therefore are not removed from the area. Only house debris eating flies are attracted and so these are a fairly good source of control. They are also good in that no visual cues are used to attract the insects. They use olfactory stimulation only and so will be effective both day and night. Light traps are only really useful in darkened rooms or at night where the light source dominates the area around the trap. The one downside to these types of traps is that there is only a limited amount of attractiveness to them due to the small area that the smell gets dispersed. They do not actively attract insects from a long distance away and are often not suitable for placing near habitation as they produce unpleasant and offensive odours.

1.8.1.4 Light Emitting Suction Traps

These have been used for many years by researchers to collect insects. The details of the work done on *Culicoides* will be discussed at a later stage under "Collection of *Culicoides* species." The traps consist of a UV light suspended above a horizontally positioned fan. When insects are attracted to the light, they are sucked in by the fan and blown downwards into a collection net or bottle. There are many pros and cons involved in the use of this type of trap. Firstly, most nocturnal insect are attracted to light and so there is no sorting of the population sampled. In the case of small fly control, a fine mesh can be placed around the whole trap thus excluding all of the bigger insects from collection. As the attractant is a light, these traps are not effective for controlling diurnal insects. They can be hung anywhere and so are quite versatile to use. They do, however, need to be plugged in and cleaned out regularly. For these reasons, they make good scientific tools for collection and may also be useful for the domestic control of pests.

1.8.1.5 Out-trapping flies to get them away from the target animals

The only way to effectively remove flies from host animals is to offer a lure that is stronger than the intended target animal. This can be done in the case to biting flies by using the cues that haematophagus flies use to find a blood meal. These are

body heat, water vapour, CO_2 and the kariomone 1-octen-3-ol. The Mosquito Magnet traps combust propane gas to produce heat, water vapour and CO_2 and the kariomone 1-octen-3-ol has been pelleted into a product called 'octenol' that is inserted into the machine. These machines produce a strong downwind plume of attractive cues that are followed by insects seeking a blood meal. The insects fly up to the trap and are sucked inside by means of a powerful vacuum fan. The plume of chemicals emitted by these traps is very powerful and has been shown by Takken and Kline (1989) to be very effective at out-trapping mosquitoes. These traps are useful because they last about one month before the gas and the octenol needs to be replaced and they catch a wide variety of blood sucking insects. The traps are sturdy and can be placed anywhere because they are self contained and do not require a plug-in point. The main downside to these traps is that they are very sensitive to the position in which they are placed. If they are not placed upwind of the target area, their effectiveness is greatly reduced. These traps are also very expensive and this fact alone precludes their widespread use.

Applications have been developed to stop insects landing on horses and to stop them biting the horses should they land. Equilline® is an application that can be used to stop biting midges and other haematophagous insects from attacking horses. Neem oil was shown by Blackwell *et al.* (2004) to effectively repel and inhibit feeding of *C. impunctis* in Scotland when applied to humans but to date no work has been done on horses.

1.8.2 Control of African Horse Sickness

To control the spread of the diseases spread by *Culicoides* species, many different approaches have been taken. Sweet itch or Queensland itch is an allergic reaction to the bites of *Culicoides* midges. Friberg & Logas (1999) tested the effect of feeding linseed oils to horses affected with this condition. Their results were that 37.2g oil /100kg horse had no statistical reduction in bites but stated that farmers and horse owners did note a reduction in scratching and irritation in the test animals.

Stabling of horses was initially thought to prevent AHS because the smell of a dirty stable would put *C. imicola* off from entering the stables. This theory was widely adopted by the horse owners community but then in 1997, Barnard found that

Culicoides imicola do readily enter stables to feed or to rest. Further investigation done by Meiswinkel *et al.* (2000) showed that *C. imicola* were two and a half times more likely to be caught in an unbaited outside trap than they were to be caught in an indoor trap in a stable containing 14 horses. He thus concluded that *C. imicola* is exophilic. From these two results it can be seen that while *C. imicola* does prefer to stay outside of stables, it will still readily enter to feed. This conclusion is backed by the evidence of cases of African horse sickness occurring in horses that were stabled at the time of infection. *C. bolitinos* has been shown to be endophilic and may well be the vector for these other cases. (Meiswinkel *et al.,* 2000). From this it can be seen that, while stabling may reduce the transmission of AHS via *C. imicola,* it may only serve to compound the problem by confining horses in an area where they are more readily attacked by *C. bolitinos.* Stables must therefore be made safe by plugging all holes and meshing the windows and doors with an impregnated mesh (Meiswinkel *et al.,* 2000).

Vaccination has proved very effective at stopping epidemics of AHS in South Africa. In the 22 years after the initial introduction of the first vaccine in 1936, only three outbreaks have occurred. A new improved vaccine was released in 1958 and from then until the present date, only three minor outbreaks have occurred (Barnard, 1998). As of 1998, approximately 130 000 doses of vaccine are administered annually in South Africa (Barnard, 1998). The drop in deaths can be seen to be related to the presence of vaccination but can also be linked to two other factors - the reduction in both zebra numbers due to over hunting, and the reduction in horse numbers across South Africa. In an effort to model vaccination of a disease with level of control, Lord *et al.* (1997) suggested that, for AHS to be eradicated, one of two scenarios must be adopted. Firstly, 90% of all horses in the region must be vaccinated against all nine serotypes of the disease or secondly, that 75% of all donkeys, and 75% of all horses receive such a vaccine. Given the cost of the vaccine and the distribution of donkeys, horses and zebra (the natural reservoir for AHS), it can be realized that totally eradication of AHS is not a goal to which South Africa must strive. Rather we should attempt to ensure thorough control of the disease by reducing the numbers of infected horses and thus keep the economically important trade in horses at a healthy level in South Africa. Given the rural location of many horses and donkeys, Rawlings *et al.* (1998) gave the suggestion that foals

be challenged with AHSV while they are still under the protection of the antibodies received from their mothers' milk in colostrum. This would of course mean ensuring that all mares are vaccinated before giving birth but would give the foals a greater chance of protection. Lord *et al.* (1997) then went on to suggest that if total eradication is not desired, then any level of vaccination will remove susceptible animals from the population and therefore will aid in reducing the disease. The complicated task of prevention was highlighted by Lord *et al*. (1996) with two hosts and one vector species . Their model was based on the Spanish experience with donkeys, horses and the vector *C. imicola*. The disease was totally eradicated through a campaign of both vector control and vaccination. Unfortunately for South Africa, we have *C. bolitinos* as a second vector and we have endemic zebra that act as a natural reservoir for the disease.

Onderstepoort Biological Products (OBP) has produced a polyvalent vaccine and while this is an effective way of controlling the disease, side effects have been noted and the temporary loss of use recommended in vaccinated horses has posed a problem to the racing and polo fraternity. While AHS poses such serious problems in South Africa, the only way of trading in horses is to first prove that they are free of the disease before shipping them abroad. This is done by segregating horses which are leaving the country in a quarantine zone for three months. This area is in Cape Town and is shown in Figure 1.2. While this system works well in theory, it does negatively discriminate against South Africa in international trade, as buyers may not wish to wait such a long period for a horse. International polo players and horse owners still prefer to incur the expense of shipping horses all the way from Argentina than waiting and paying for the long quarantine process in South Africa. Unless AHS is far better controlled, no change to this situation will occur.

1.9 Collection of *Culicoides* species

To understand the ecology and interactions of the vectors, they must first be found, caught, and studied. Collection of these wild midges is an area of huge knowledge. There are many traps that have been used. See paragraphs 1.8.1.1-5. Some traps are very elegant, such as the Haufe-Burgess mosquito trap that separates catches

hourly (Southwood & Henderson, 2000) and other trap designs are simple, such as the sticky tape traps that can be bought in supermarkets. In South Africa, a standard down-draught suction trap (Figure 1.10) has been used extensively. An 8W UV globe is used as the attractant and this is mounted above a fan that creates a down draught of air into a collecting bottle. Usually the entire trap is covered in 2mm mosquito gauze to stop any big insects from contaminating the samples. Usually the traps are powered by a 240V electricity supply (Van Ark & Meiswinkel, 1992; Barnard, 1997; Musuka *et al.,* 2001; Meiswinkel & Paweska, 2003; Rawlings *et al.,* 2003,) and occasionally they are powered by a 12V supply or less (Rawlings *et al.,* 1998, 2003). The UV light is used because of the nocturnal and /or crepuscular nature of the midges (Murray, 1991).

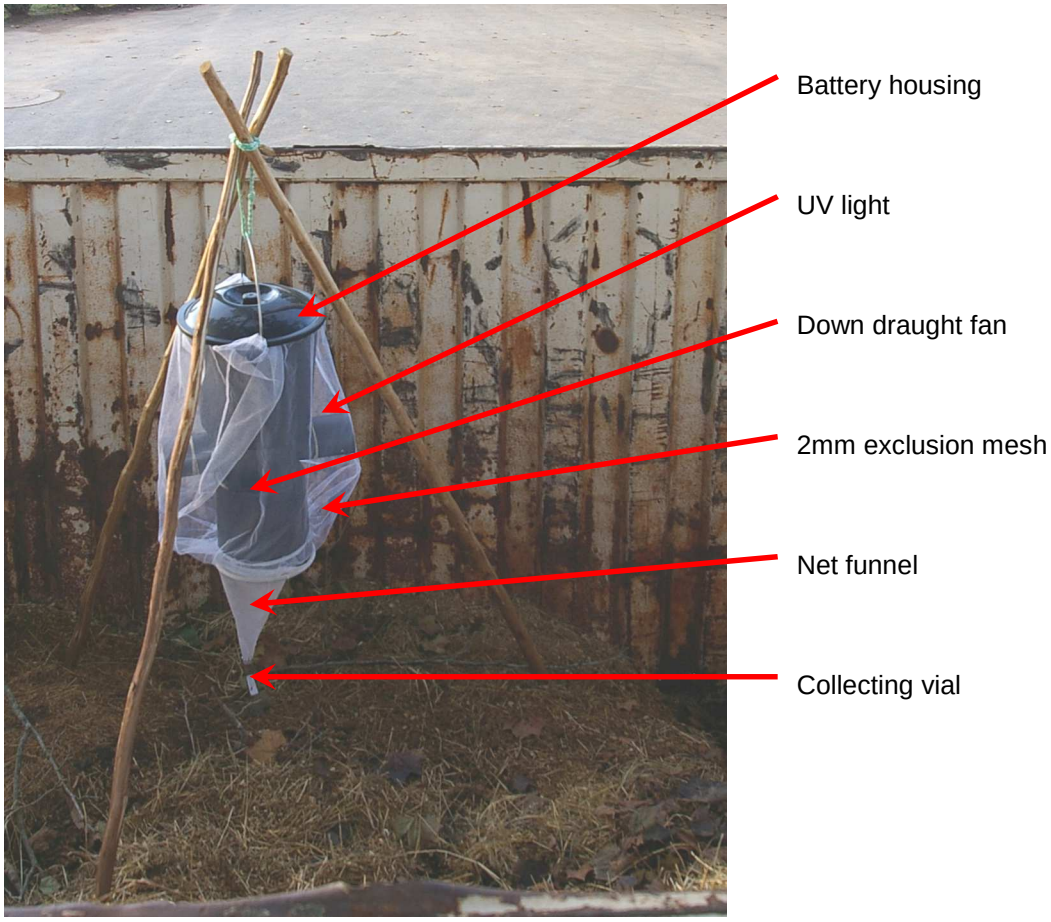

Figure 1.10 A standard downdraught fan for the collection of *Culicoides* species.

The traps of Holbrook & Bobian (1989) had solar powered batteries and light intensity switches on them so that they turned themselves on at sunset and off at

sunrise. Midges are lured in using the UV light and then sucked into a collecting bottle containing either a phosphate buffered saline solution to which a few drops of Savlon are added, or 70% alcohol solution. The saline solution is useful if the midges are to be later used for virus assays such as ELISA or PCR tests and the alcohol is used if the specimens are to be either slide mounted or stored for long periods. The effectiveness of these is shown by a catch of approximately one million individuals in one trap in one night by Meiswinkel (1989) in the Onderstepoort area. Bishop *et al.* (2004; 2006) tested the effectiveness of using light emitting Diodes (LEDs) as an attractant. They found that green light was the most attractive colour followed equally by blue and white, followed by the control (light bulb incandescent light) and yellow and finally red, which was the least attractive to *C. brevitarsis*. They suggested that green light be used for the collection of midges even where populations are sparse and that red light be used instead of incandescent light if night work needs be done on cattle and sheep.

Light traps work extremely well at attracting positively phototactic insects. Fortunately all ceratopogonids fall into this category. Many traps also use carbon dioxide as an attractant. This can be produced in the trap as in the Mosquito Magnet and the Italian made ActiPower Trap or it can simply be produced by hanging dry ice in paper bags next to the light. This is useful as it has been shown to increased catches of some midge species but was also shown to actually repel female *C. variipennis* (Holbrook & Bobian, 1989) when used with light. Traps such as the Mosquito Magnet also have an added kariomone, octenol, in them which improves catches (Takken & Kline, 1989; Kline *et al.,* 1994; Ritchie *et al.,* 1994). These traps are excellent for catching females of the species that are attracted to CO_2 and kairomones but are not effective at trapping a representative portion of males. For this reason, Kettle *et al.* (1998) used a truck mounted trap that was driven up and down a set pathway at certain times. Johansen *et al.* (2003) used truck traps, huge land nets, and a kytoon, which is an aerial balloon mounted trap set at between 75 and 150 meters to sample wind borne insects. Blackwell (1997) used suction traps so that light or attractants would not skew her collections. She also used sticky boards initially but discarded the practice as the small insects were impossible to identify when covered in glue. Kettle *et al.* (1998) found that the truck trap was far inferior to light traps at a rate of 1:27 individuals caught. Light traps

were also shown by Meiswinkel *et al*. (2000) to not totally override animal bait attractants and so is a good method to use when collecting near livestock. Midges will first be attracted to the animals and then caught by the trap.

1.10 Research methods used with *Culicoides* midges

Once collected, most samples are identified to species using keys to the wing pictures of the species (Figure 1.11) and then sorted into males and females. The females are then separated further into any number of possible categories. Murray (1991) used 6 categories to classify his catches of *C. brevitarsis*. They were separated into nulliparous, blood fed nulliparous, empty parous, blood fed parous, parous, and gravid parous. Each classification gives an indication of the population structure in the area and so is important to know. When huge numbers are collected, subsamples are taken so as to determine to total population of midges. The statistics of various subsampling techniques have been tested and show that when sampling a large catch, sub samples should be at least 500 individuals in size and should be done on a mass-of-midges basis rather on a volumetric basis of midges suspended in alcohol. Cleaning of the catches to remove non-*Culicoides* species is only necessary if there is a large proportion of other insects that are significantly bigger than the *Culicoides* midges (Venrick, 1971; Van Ark, 1975; Van Ark & Meiswinkel, 1992). This method allows an accuracy that falls within the acceptable 10% sub sampling error defined by Southwood & Henderson (2000) for ecological studies of invertebrates.

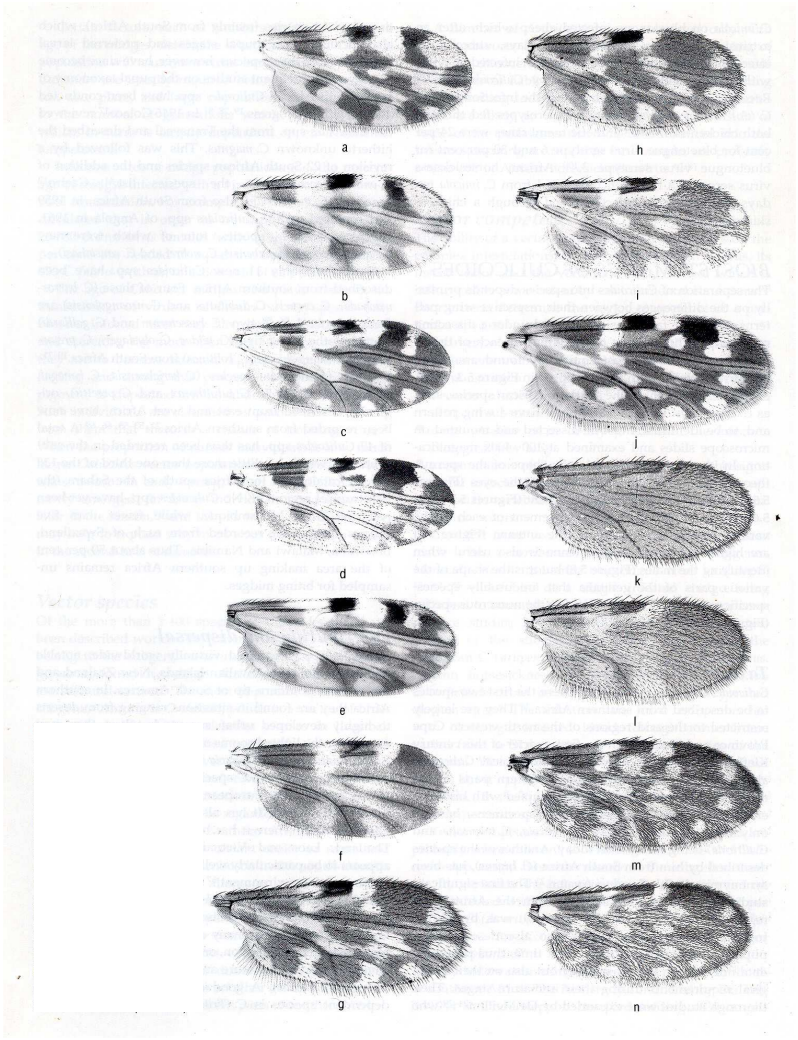

Figure 1.11 Wing picture of 14 species of South African *Culicoides* (Venter & Meiswinkel , 1994) .

All of the above collection techniques are for the flying adults of the species. Larval habitats are also of crucial importance if larvicidal applications are to be studied. The most common practice for collecting larvae is to cut samples of habitats from the environment and then return them to a temperature and humidity controlled insectary, where they are given optimum conditions for a minimum of three weeks at which to develop and emerge (Nevill, 1968; Braverman *et al.,* 1974; Lubega & Kahmala, 1976). Emergence chambers are set up whereby the emerged adults are attracted to a light source and then collected either by falling into a liquid suspension or by simply being aspirated into a bottle by researcher (Mellor & Pitzolis, 1979). Samples of the habitat need only be at the most 6cm deep as *Culicoides* larvae

reside in the top 3 cm of soil. For dung dwelling species, either whole cow pats are marked, left in situ for 2 days to allow colonization by midges and then set up in emergence chambers (Bishop *et al.,* 1996) or cores are cut using a piece of PVC piping and used as representative samples (Bishop *et al.,* 1998). To obtain absolute numbers of larvae from a sample, flooding the sample with salt water has been shown to cause most larvae to float and they can thus be collected from the surface. This does however kill many species as larvae both drown and desiccate if salts and water content in their habitat are too high. Nevill (1967) showed that the larvae of *C. imicola* drown and do not float to the surface. This method therefore can not be used on this species.

If a total understanding of the life cycles of midges is to be obtained, viable lab populations must first be set up. Despite the efforts of Edwards (1982) on *C. subimmaculatus* and Nevill (1967) on *C. imicola*, no viable laboratory population of South African *Culicoides s*pecies has yet been established. Kitoaka (1982) mentioned a colony of the kokubunji strain of *C. arakawae* that had run since 1977, which was one of only a few such colonies to have been cultured *in vitro* successfully. To observe life cycles of local populations, wild caught midges are first either anaesthetised with CO_2 (Nevill, 1968) or chilled to -3ºC (Edwards, 1982). The inactive midges are then returned to the lab where they are sorted into individual vials containing a piece of moist filter paper on which to lay their eggs. Most midges will lay the first batch of eggs autogenously, that is without a blood meal, but those that need a blood meal are fed either on the researcher (or assistant), test animal (Nevill, 1968; Fahrner & Barthelmess, 1988) or via a membrane technique using either a latex membrane filled with blood (Fahrner & Barthelmess, 1988) or the skin of a day old chick. Eggs can be obtained simply by decapitating the females. This causes a survival reaction and the eggs are usually laid within a few hours of decapitation (Kitaoka, 1982). The egg-laden filter papers are then placed in a suitable environment for the rearing of the larvae. Nevill (1968) placed the papers on a slope of soil which was flooded at the bottom and dry at the top. This allowed the larvae to migrate up or down the slope to find their optimal moisture zone. Edwards (1982) used agar with a sample of the collected habitat, while Kitaoka (1982) went used an agar substrate and fed his larvae with a suspension of nematodes to ensure optimal growth.

The work of Bishop *et al.* (1998) has shown that for maximum emergence rates, larvae of *C. brevitarsis* reared from dung should be kept under constant light conditions rather than a light/dark regime, and Bishop *et al.* (1996) showed that for *C. brevitarsis* the best temperature at which to emerge adults from pupae was 25ºC to 28ºC. They also showed that adults reared at only 12 ºC were one-third the size and far less fecund than those reared at higher temperatures. Kitaoka (1982) showed that the best temperature of survival and pupation of C*. arakawae and C, maculates* was 22.5ºC and that lower temperatures induces high fecundity and bigger females (what he termed as ´might´ midges), but high temperatures had the reverse effect. Carpenter *et al. (*2006) found that oogenesis (egg development) was also affected by temperature and that it took female *C. impunctis* 156 hours to develop 50% of their follicles at 15ºC but only 108 hours at 30ºC. In an engorgement site experiment done by Townley *et al* (1984) it was found that various midges in Ireland prefer to bite at on the lower leg regions and along the mane.

1.10.1 Statistics for data analysis

All arthropod response variates are usually highly variable and it is for this reason that most data is log transformed prior to statistical analysis (Holbrook & Bobian, 1989; Kettle *et al.,* 1998; Bishop *et al.,* 1998, 2004). If significant differences between treatments are required, Blackwell (1997) and Bishop *et al.* (2000) used a standard Students T- test.

1.10 Conclusion

African horse sickness is a devastating disease of equines in enzootic and epizootic incidences. Research is required to ascertain ecological implications of a changing weather system, as life stages of the midges are so highly correlated to environmental variables. Predictive models are useful in that they highlight when the next large vector population will occur but the existing models need to be globally applicable. Prophylactic control strategies should be enhanced in cogniscance of the larval and adult stages of the midge and the habitats and preferences they display. Vaccination should be mandatory and extensively carried out. So too should vector control using both larvicidal and adulticidal formulations.

CHAPTER 2: A NEW, REVOLVING LIGHT TRAP DESIGN FOR TESTING COLOURED LIGHT PREFERENCE OF NOCTURNAL INSECTS

A.B. JENKINS[1] and R. HENDRIKS[2]

2.1 Abstract

A new, electrical, revolving, trap designed to test preference to light colour and source in small nocturnal flying insects is described. Specifically aimed at testing *Culicodes* African Horse Sickness Vector preference for green, white and UV lights emitted from Light Emitting Diodes (LEDs) or fluorescent tubes, the light trap uses oil coated Perspex sheets in front of the lights to capture small insects. It is powered by mains electricity and power is equalised across the different bulbs so as to give an indication of which is more efficient at attracting midges. Catches are semi-sorted with large insects escaping and small insects remaining firmly stuck to the oily film.

2.2 Introduction

South Africa is the enzootic range for African Horse Sickness (AHS) Virus (Mellor & Hamblin, 2004). The virus is transmitted by ceratopogonid flies of the genus *Culicoides* and the two species proven to be the vectors of AHS are *Culicoides imicola* and *C. bolitinos* (Du Toit, 1944; Venter *et al.*, 2000; Paweska *et al.*, 2003). Despite a large scale vaccination plan that is in place in South Africa, the yearly losses are still great (AHS, 2006). Midge control is an important aspect of the control of this disease and if the vectors are to be studied effectively, one needs the most efficient trap available. The two species in question have both been shown to be crepuscular (Barnard, 1997; Meiswinkel *et al.*., 2000) making dusk to dawn traps effective. Many studies have used standard 8W Ultra Violet (UV) fluorescent light downdraught traps (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Musuka *et al.*, 2001; Paweska *et al.*, 2003; Rawlings *et al.*, 2003) as UV light is attractive to almost all night flying insects.

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Mellor & Hamblin (2004) suggested that UV blacklight traps be used as a standard means of monitoring *Culicoides* midge populations. Bishop *et al..* (2004) found that certain species of midges were preferentially attracted to green and blue light. In a further comparative trial by Bishop *et al..* (2006), incandescent light and five different coloured Light Emitting Diodes (LEDs) were tested with the final result being that red light was very unattractive and green light the most attractive to *C. brevitarsus, C. bundyensis, C. nattaiensis* complex and *C. victoriae*. However, three other species were found to be preferentially attracted to UV LEDs. LEDs were also found to produce a greater general attraction across all collections than incandescent light, the Australian standard. The South African collection standard of UV fluorescent lights was not tested. The comparison of the South African standard and any other type or colour of light, has not, to date, been tested locally.

The one difficulty in testing light preference of insects is that of confounding experimental design. If two different light traps are erected side by side, it can not be determined which was the more effective lure and to what degree this was so. Baffles can be erected between the traps but these only serve to further complicate the issue as now one of the two traps is shielded from half of the available collection habitat. If the traps are placed either side of a stable or around the corner of a structure, the same is true. How can the greatest attractant be determined? Multiple collections across many nights have been used to solve the problem as pseudo-replicates can be collected from each site. This method is not, unfortunately, entirely impartial. Insects tend to congregate around a certain environmental structures at specific climatic conditions which may change from night to night, for example, insects may alight on the downwind walls of a building. In an area with fluctuating wind direction, this may predispose the downwind colour to catch more insects. This can only be solved by repeating the nightly collections a great many times and thus minimising the environmental influences. Venter & Hermanides (2006) chose to place traps “10-15 meters apart at opposite ends of a stable housing 15-20 horses.” Bishop *et al..* (2004) chose to run their trial on a larger scale, erecting traps along a fence line approximately 20 meters apart. Only four collections were made with traps being randomly assigned a new position at each collection. Environmental conditions, trap placement and interference between traps may all influence the repeatability of results.

If we are to improve the efficacy of traps to catch adult AHS vectors, different light sources and colours must be tested and critically evaluated, and to this end, a new revolving trap was designed. It compares the catch yields of midges attracted to LED and fluorescent light sources of a variety of colours. The results of the use of this trap can be used as a basis for the development of a more effective sampling tool with which midge populations can be sampled.

2.3 Materials and Methods

2.3.1 General features of the trap

A 70cm four-sided box was constructed out of perspex, aluminium and electrical circuitry. White, green and UV lights of both LED and fluorescent tubes were mounted vertically on three of the four sides. The trap was set to rotate once every 3.5 minutes, slow enough to facilitate a side selection for even the weak flying *Culicoides* midges (Walker, 1977; Blackwell, 1997).

Three light colours were chosen for comparison, white, green (Bishop *et al*., 2004; Bishop *et al*., 2006) and UV (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al*., 1998; Musuka *et al*., 2001; Paweska *et al*, 2003; Rawlings *et al*., 2003). Power to the box was supplied via 240V mains electricity. An equivalent current was given to each type of light and it was noticed that the light from LEDs was more directional and intense than that from the fluorescent lights.

The light panels were covered by a clear perspex plate sprayed with a commercial vaporised oil (Spray and Cook). The oil served to detain small insects without structural damage. Species identification of midges relies on wing patterns (G. Venter, *pers. comm*. 2006) making it imperative that they were not damaged, as would occur with adhesive alternatives to the oil.

At dawn, catches were transferred to acetate for cleaning and then preserved in 80% alcohol for identification.

The primary features of the design are:

- No structural damage of catches
- Equal power to light sources
- Comparison of LED and fluorescent light sources
- Comparison of colours
- Capacity for daily collections
- Slow rotational speed to prevent wind / habitat and locational bias

The confounding aspect of having two lights visible at once was partially overcome by recessing the panel housing of the light bulbs on the box. The small sides afforded some degree of protection but did not completely block out each light. Baffles were considered but were omitted as they would have made the trap highly susceptible to wind damage should it have been blown over. Figure 2.1 shows this slight recession of the light panels

2.3.2 Technical details of the trap construction.

2.3.2.1 Box

Figure 2.1 shows the stages of the box construction. Grey plastic panels measuring 275mm X 630mm X 6mm were milled out, for LED clusters and fluorescent mountings, and slid together in an interleaving fashion. They were tensioned using threaded bar that ran the length of the trap. This box formed the fundamental rectangle onto which the clear perspex panels could be installed. The clear panels were held in place using aluminium angle iron brackets that were riveted onto the sides of the panels

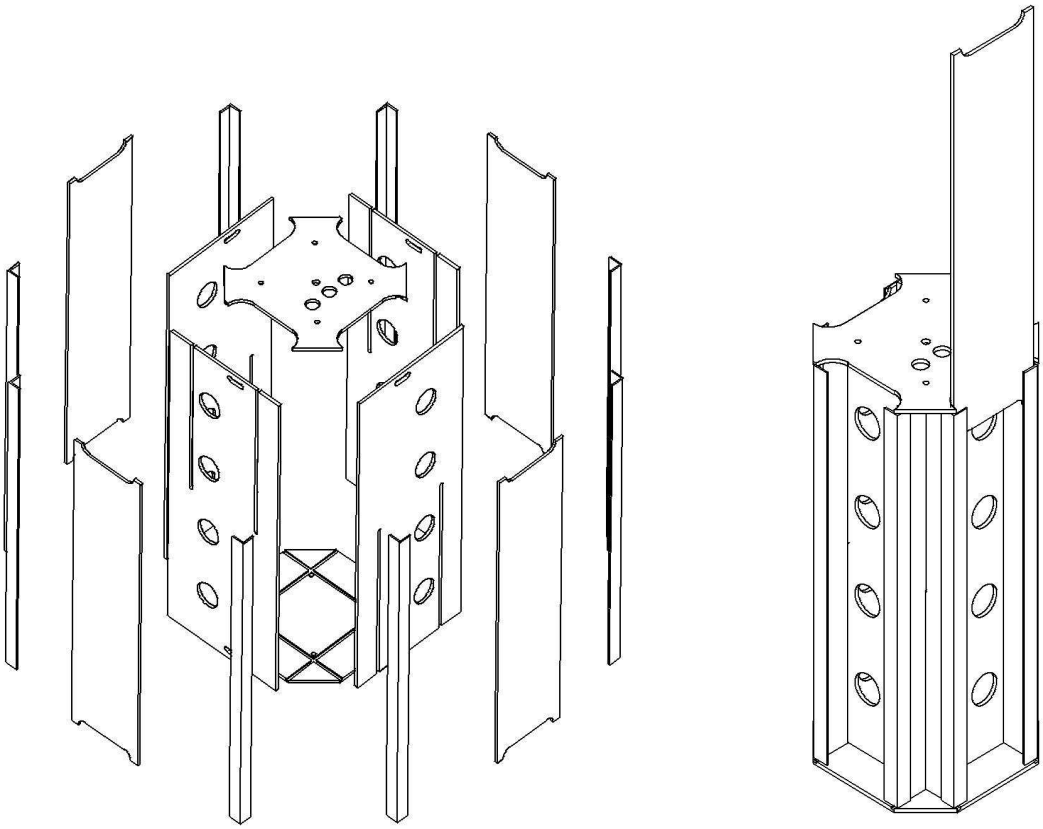

Figure 2.1 3D design diagram of the trap box. All individual panels interleave to form the finished box and the clear perspex collection panels.

The control face (side 1) of the trap contained no light sources on the grey plastic panel under the clear perspex. Sides 2, 3 and 4 included a single fluorescent tube and four circular clusters of LEDs in white, UV and green respectively.

Panels were wired individually (Figure 2.2) to supply power to lights on that side of the trap and control the light intensity.

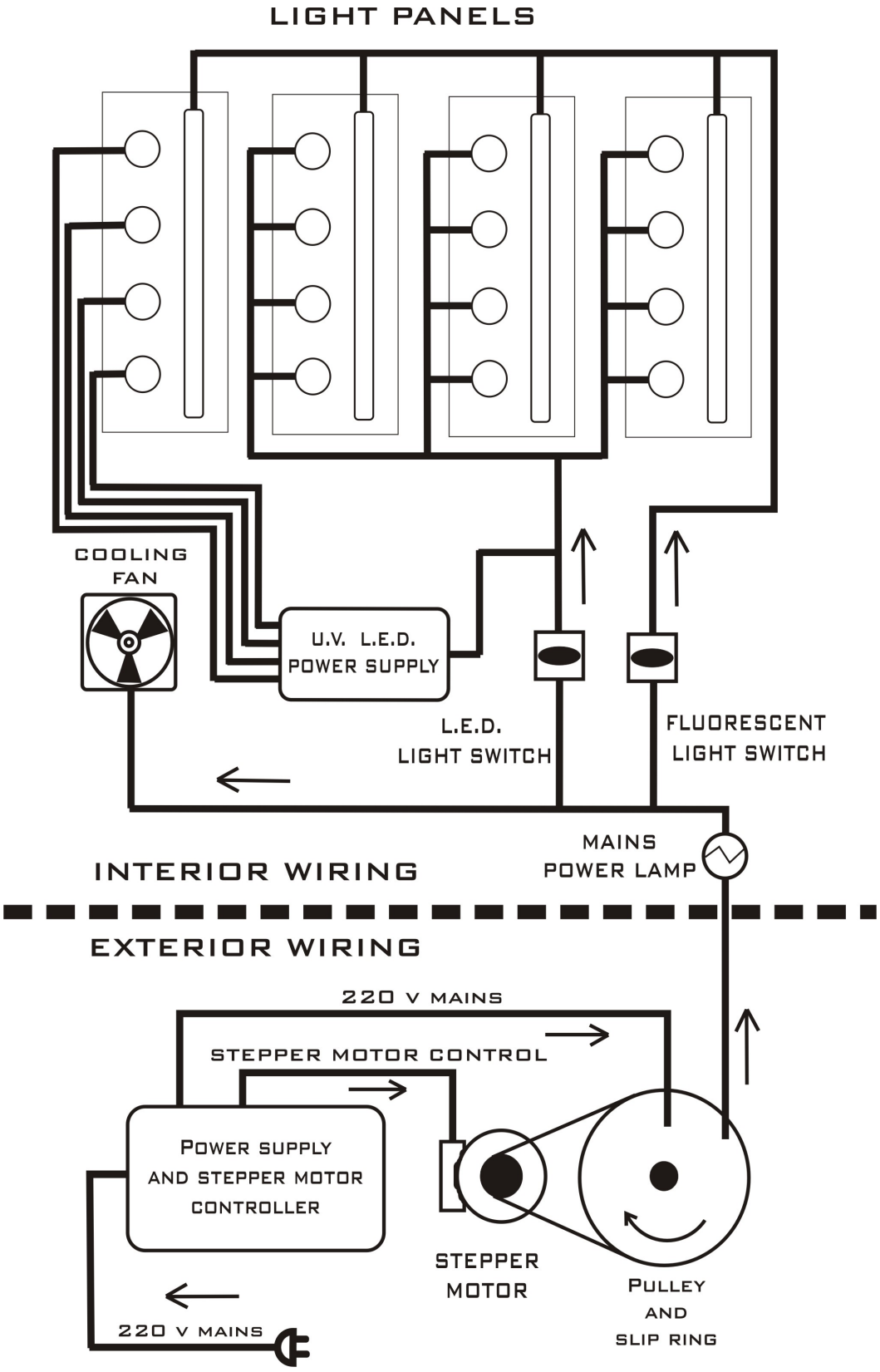

Figure 2.2 Electrical diagram of the trap.

2.3.2.2 Lights

Exterior 220V mains power through a control box, stepper motor, and slip rings supplied power to the interior to the trap. The three different types of light bulb on the trap and the defined rotation (1 rotation every 3.5 minutes) necessitated transformers to modify the 220V mains current.

UV LEDs had to be constructed by soldering LEDs onto yellow board. A separate transformer was mounted to drop the 220V to the required 8V (Figure 2.3). White and green LEDs are available as complete light bulbs with their own power converters and stepping down transformers to provide their 12V requirement. The fluorescent lights required 220V power. The LEDs and power supply to the fluorescent tubes were all mounted on the interior surface of the panels (Figure 2.4). The green and white sides were wired as such.

A small red indicator light showed that the trap had power and was situated between separate switches for the LED lights and the fluorescent lights which could be turned on separately or simultaneously.

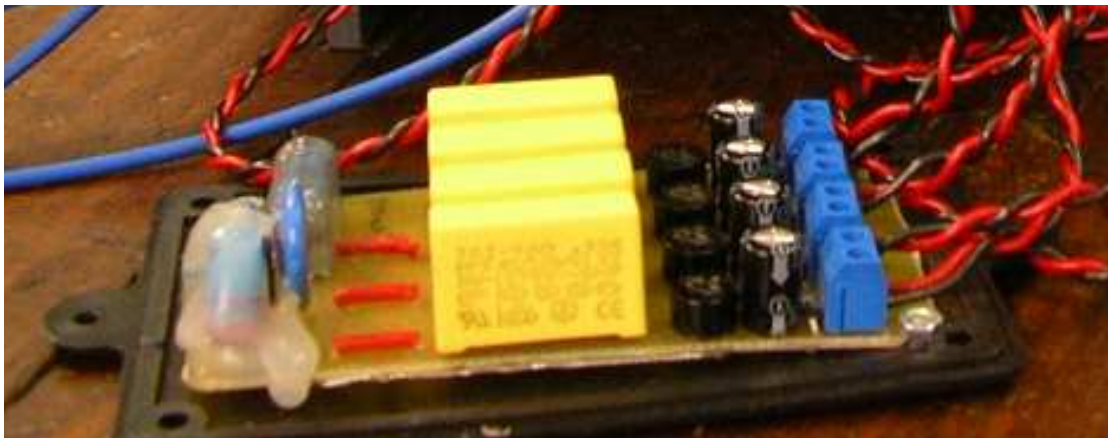

Figure 2.3 Power supply for UV LEDs. This device drops the current from 220V to 12V and supplies power to all four UV LED clusters.

Figure 2.4 LEDs and power supply to the fluorescent tubes were all mounted onto the internal surface of each panel.

2.3.2.3 Cooling

Having so much electrical equipment in the small interior caused a lot of heat to build up. An 80mm diameter muffin fan was installed in the base of the trap to supply cool air to the trap interior. Three large holes cut into the top panel facilitated the release of hot air. Although without lights, side 1 (control) had the holes for the LED mountings, without the lights installed, to provide some lateral ventilation as well.

2.3.2.4 Turning Mechanism

The trap needed to be rotated at a desired speed, as slowly or as fast as required, with a very high and consistent torque so that environmental conditions would not affect the speed at which it turned. A small stepper motor was selected and connected via an 8:1 cog ratio to the top axel of the trap (Figure 2.5). Power was provided to the trap via a splitter box which supplied the lights, fans and motor with their required voltage. Electrical current was directed into the trap through a series of brass and graphite slip rings so that the trap could continuously turn. Figure 2.5 shows the detailed mechanics of the turning mechanism.

2.3.2.5 Overall Trap Construction

The frame was made of angled aluminium bar and is 800mm high and 500mm wide and deep. The trap legs are 100mm long and allow the trap to be slightly out of the ground level dust. A bent, 2mm thick aluminium roof keeps rain and sun off the Perspex box and circuitry (Figure 2.6). This simply tensions firmly onto the frame.
A supporting cross beam at the base of the trap (Figure 2.6) stabilises the turning box.

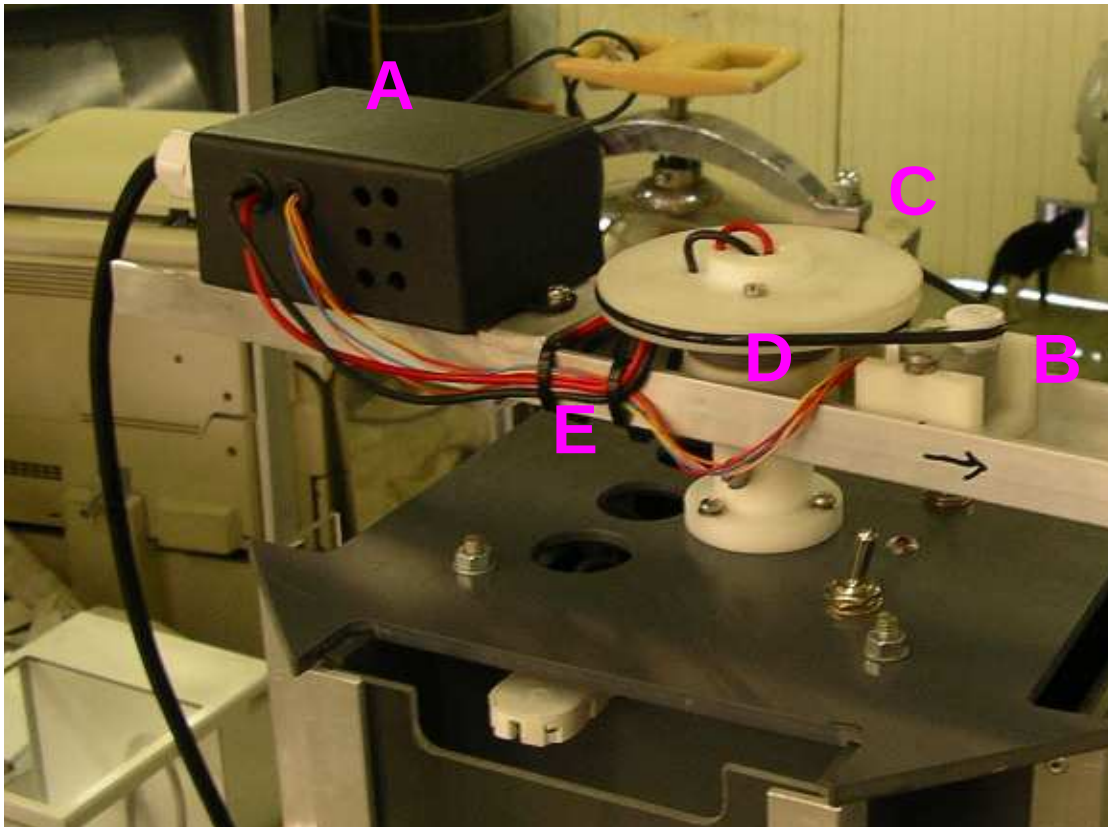

Figure 2.5 Photograph of the top assembly of the trap showing the splitterbox (A) , stepper motor (B), 8:1 conversion ratio wheels (C), brass and graphite slip rings (D), and ventilation holes (E).

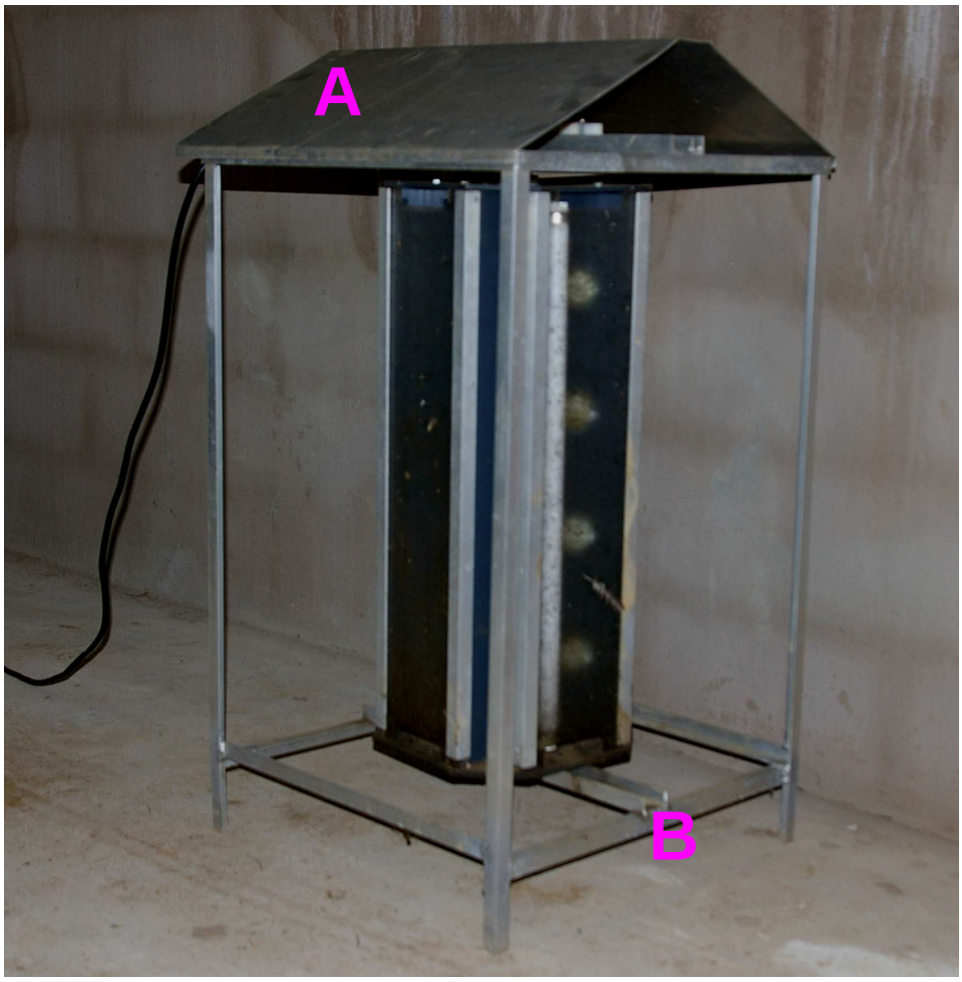

Figure 2.6. Photograph of the completed trap. Note the aluminium roof (A) tensioned to the frame and bottom cross beam supporting the spindle that stabilizes the turning box (B).

2.4 Results and Discussion

The prototype was successfully tested in front of one of the Spurwing stables in Karkloof in the Midlands of KwaZulu-Natal (GPS Ref E30°14'43.3" S29°22'47.9" elevation 1116m). The results of the subsequent trial appear in Chapter 6. The trap was run for 15 consecutive days with 8 nights using the LEDs and 7 nights using the fluorescent light. A total of 3727 *Culicoides* midges were captured with nearly 5 times more being caught with the LEDs (3025) than were caught with the fluorescent light (702).The nightly average catches from the individual light colours

were: green LED (77), UV LED (264) and white LED (91) and green fluorescent (29), UV fluorescent (46) and white fluorescent (47).

The trap was made out of hard plastics and an aluminium frame and roof. This gave it a lot of strength and enabled it to withstand a good deal of interest from large animals. Horses were seen chewing and pawing the trap with no ill effects.

The legs of the trap are 10cm long and at the stable where the trap was tested, a pair of large pigs roamed freely. Because of the short legs of the trap, they were able to reach the oily sheets and licked off many off the insects during the night. Also, a lot of dust was blown onto the bottom portion of the trap. Both of these problems were overcome by simply putting the trap on a small camping table.

The sturdy construction and the use of oil as the capture medium were tested during periods of strong wind and driving rain. The trap withstood the environmental conditions with no damage and the oily film was water repellent and none of the trapped midges were washed off the sheets. This was confirmed because no insects were found on the base of the trap or the concrete floor under it.

Spray and Cook as the capture medium was very good for many reasons. The oily film was not sticky but rather permanently wet. This allowed a simple sorting of the catch into larger insects, which hit the sheet and then flew away, and the smaller insects which remained firmly stuck to the oil. Figure 2.7 shows four midges trapped to the oil film of the Perspex sheet. Large patterns of wing scales were seen on the sheets indicating that large insects were not captured by the trap. However, it was noted that these large insects did not dislodge any of the smaller ones as there were often midges trapped under the patterns of wing scales. Using the oil also meant that the catches were easy to sort as insects were trapped as they encountered the trap and not all bunched together in a vial. The use of the oil could also be used to judge the flight height of small insects as it was noticed that there were generally more insects on the top of the sheets than the bottom. The use of oil and the release of larger insects negated the need for a fine mesh around the trap. Many light traps for *Culicoides* spp. are surrounded by fine mesh gauze to stop other large

insects from contaminating the catches. This could act to reduce the catch sizes of these traps as the midges alight on the mesh and then must actively crawl through the mesh before being caught. With the oil, this is not the case.

Figure 2.7. Four *Culicoides* midges trapped to an oily sheet. Removal simply entails gently picking them up with tweezers.

The trap was very easy to set up and was very reliable. As all of the mechanical components are housed directly under the roof, no problems were experienced with the trap. The trap can be given to a farmer and set up by him provided that there is access to a 220V power supply.

Recent work has shown that midge activity at different areas around a stable is highly variable (Author's unpublished data). This variability makes replicate collections very difficult. For example, large differences in midge numbers were noted from collections from the front and from the back of a stable block. Interference between traps is a major issue in insect collecting experiments as is

geographical or climatic conditions and pseudoreplicates. Statistical design of the experiment provides options where lights and rotation preclude the confounding in the design. By placing the colours on a single trap and then revolving the whole structure, all problems of preferential habitat sampling and interference are solved. This trap allows for true replicates to be done. If two panels are set to one colour and two to another colour, then duplicate readings are taken each night this would allow the data to be far better analysed statistically than the current pseudo-replicates supplied by sampling in different locations, or over different times.

The flexible design of the trap means that changes can be made according to the type of trial required. The lights can be modified to accommodate strobing or slow flashing. Combinations of colours can be tested. The speed at which the trap is rotated can be changed making it rotate as slowly as required. The speed of one turn every 3.5 minutes was almost imperceptible.

The oil on the surface of the collecting sheets must be replaced each day. It took approximately 48 hours to form a hard layer from which it was difficult to remove small insects without damaging them. A light cooking oil was also tested. The oil stayed wet for up to four days and was easier to get off the sheets at the end of each nights' catch. The oil seemed to be just as effective as the Spray and Cook at catching midges.

Tweezers must be used to remove the insects and this can be damaging if the technician is not careful. A fine paintbrush could be used to replace the use of tweezers but this was not tested. As *Culicoides* midges are very small, a 10X dissecting microscope was taken into the field. This method was cumbersome but, once the collector is used to the process, the midge removal can be done by eye.

The trap was originally designed to catch *Culicoides* but it is equally effective for catching a variety of nocturnally active Arthropoda, e.g. Lepidoptera, Coleoptera, Hemiptera, Diptera, Hymenoptera, Ephemeroptera and Trichoptera.

2.5 Conclusion

While results of the trial are discussed elsewhere (Chapter 6), this design experiment has provided an effective means of trapping *Culiciodes* and other nocturnal arthropods. The combination of fluorescent and LED light sources and a slow rotation provides a cost effective diagnostic tool that not only prevents location and geographical discrimination but is also sturdy and durable. The use of this trap provides a means of comparing the influence of light, light intensity, speed of trap rotation, flight height, species, location, weather and season on the yield of crepuscular arthropods. Particularly effective in trapping *Culicoides* spp. midges, it offers an effective means of evaluating the most effective prophylactic light strategy in the fight against AHS.

CHAPTER 3: A COMPARISON OF COMMERCIALLY AVAILABLE MOSQUITO TRAPS FOR THE CONTROL OF *CULICOIDES* MIDGES.

A. B. JENKINS[3] and G. J. VENTER[4]

3.1 Abstract

Three commercially available, propane combusting, pheromone baited traps were tested at a farm in KwaZulu- Natal to determine their efficacy at out-trapping *Culicoides* midges, the vectors of African Horse Sickness. The traps were run for 20 days at 10 sites around a stable block housing 6 horses. Catches were sorted, identified and data log(X+1) transformed. This transformed data was statistically analysed using the ANOVA test. Proportional catch ratios were compared using a Z test method. This style of trap was shown to preferentially catch more nulliparous and parous females than blood fed and gravid. No one trap was any better than another at trapping midges. All catches across the 20 days were extremely small leading to the conclusion that this style of trap is ineffective at controlling midges in KwaZulu-Natal.

3.2 Introduction

The control of all flies is a difficult task to undertake as they occur in huge numbers and often reproduce faster than they can be killed. Many methods of control are available. These include spraying insecticides over an area, modifying their habitats to reduce possible breeding sites and trapping the adult insects. As discussed in Chapter One, of the many methods of trapping insects, the only method that is fairly specific to blood seeking insects is that of using specialist commercially available machines to effect control on a population.

Culicoides imicola and *Culicoides bolitinos* are proven vectors of African Horse Sickness (AHS) in South Africa. Due to the drastic interventions being used against these AHS vectors, it was decided that a test of these commercial traps was needed

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to determine whether they were effective at controlling the numbers of midges around stables where horses were kept.

Adding a chemical stimulus to traps in order to increase catch rates of blood seeking insects has been highly productive and has paved the way for the conception and manufacture of these traps. Gillies & Wilkes (1972) showed an attraction from as far away as 15 meters to CO_2 bait. Gillies (1980) showed that CO_2 had a definite orientation effect on the flight of moths and mosquitoes with the test insects' flight being linear up a concentration gradient of CO_2 and laterally random when there was no chemical signal. Holbrook & Bobian (1989) showed that the CO_2 emitted from bags of dry ice hung next to a trap raised the catch rate of blood seeking insects. From these results, the conception of a CO_2 emitting trap was born. Gillies (1980) also showed that CO_2 is far more effective as an attractant when the gas is released as pulses rather than a continuous stream. He concluded that this was because the pulses of gas and the changing concentrations in the air stream accurately simulate the natural turbulence that surrounds any warm blooded animals. Other chemical stimuli have also been shown to be attractive to blood seeking insects specifically. 'Ox Odour' was used by Vale & Hall (1985a; 1985b) and was broken down into its constituent parts. Contained in this odour were acetone, lactic acid, and a compound called 1-octen-3-ol (henceforth called octenol). The idea of an additive, or synergistic, reaction between CO_2 and host animal odour was uncovered by Vale & Hall (1985 a) during their work with tsetse flies. Figure 3.1 shows the effects of adding CO_2, acetone and octenol together to produce a marked attraction response in the flies.

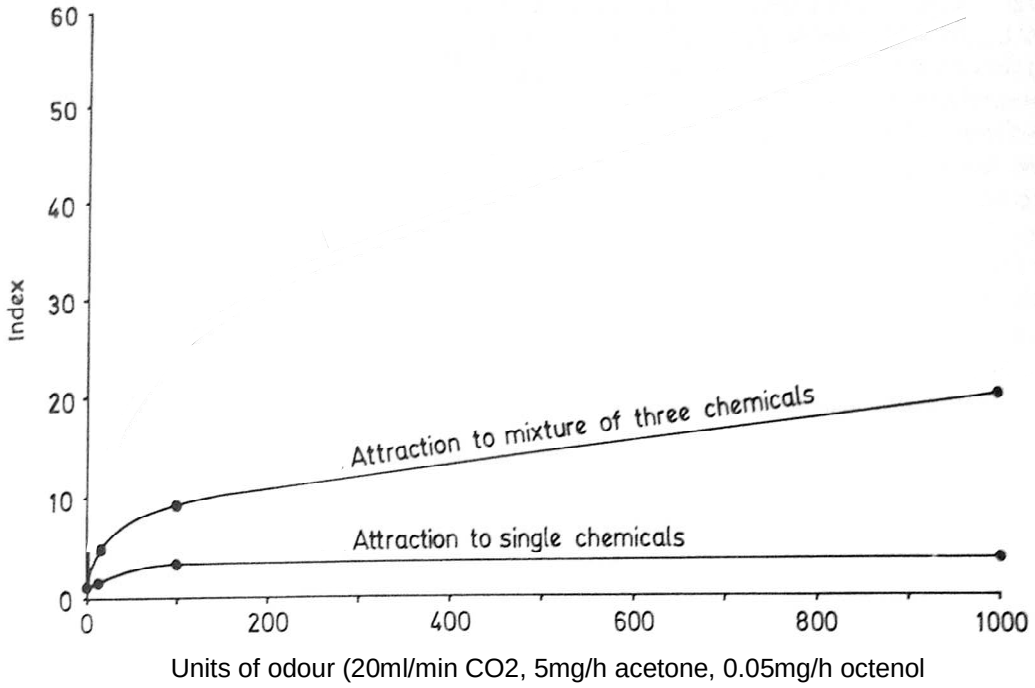

Figure 3.1 Generalised relationship between the index of response of *Glossina* sp. and the dose of various odours (taken from Vale & Hall 1985b).

Takken & Kline (1989) also showed that a CO_2 emission rate of 200ml/min significantly raised catch rates of a host of haematophagus insects including Ceratopogonids and catch rates of both mosquitoes and ceratopogonids have been shown to be significantly raised when octenol was added to CO_2 baited traps (Kline *et al.,* 1994; Ritchie *et al.,* 1994). It was work like this that produced the pelleted form of octenol available for use in these commercial traps.

Not all insects are attracted to CO_2 and octenol to the same degree and some may even be repelled by the CO_2. In work done on *Culicoides hollensis* and *Culicoides melleus,* Kline *et al.* (1994) found that the addition of octenol to a CO_2 baited trap actually repelled the midges and had absolutely no effect on several other species. In the face of such conflicting research, broad generalisations can not be made and it is highly unclear whether the commercially available traps described above would be a useful tool with which to control *C. imicola* and *C. bolitinos* in KwaZulu-Natal, South Africa. It is the aim of the experiment to place all three traps under identical

conditions and determine if they are a choice for equine enthusiasts who wish to control AHS vectors on their farms.

The machines that are currently available in South Africa are the American produced Mosquito Magnet™ machines (Figure 3.2) that come in a range of sizes and power ratings, and the Italian produced Actipower Trap™ (Figure 3.3). All operate on the same principle but show variation in a few details.

Figure 3.2 Two differently sized Mosquito Magnet™ traps. "A" is the Mosquito Magnet Liberty Plus, capable of generating a plume to cover 1 acre, while "B" is the smaller Mosquito Magnet Defender, capable of generating a plume covering 0.5 acres (Mosquito Magnet, 2007)

Figure 3.3 The Italian produced Actipower Trap™ with the additional feature of a light source as an attractant (Actipower, 2007).

The principle that the machines all act on is one of reproducing all of the chemical cues that mosquitoes use to get a blood meal. These are body heat, water vapour (Gillies, 1980), CO_2 and the octenol. All of these traps combust commercially available domestic propane gas to produce heat, water vapour and CO_2. 1-octen-3-ol has been packaged[5] into a 55.15% strength commercial product called Octenol™ for the Mosquito Magnet™. The Actipower Trap™ does not use octenol but rather a product named ActiVix™ which is composed of lactic acid, ammonia and other carboxylic acids[6]. Both products are inserted into their respective machines and release a controlled amount of the substance into the strong downwind plume of attractive chemicals. This plume is very concentrated and as it is blown over any animals and people, it floods the environment thereby confusing blood seeking insects and attracting them away from the potential hosts. Haematophagus insects seeking a meal are attracted to the trap and are sucked inside by means of a powerful suction fan (Figure 3.4).

[5] Octenol ™ package insert, 2006.
[6] ActiVix ™ package insert, 2005.

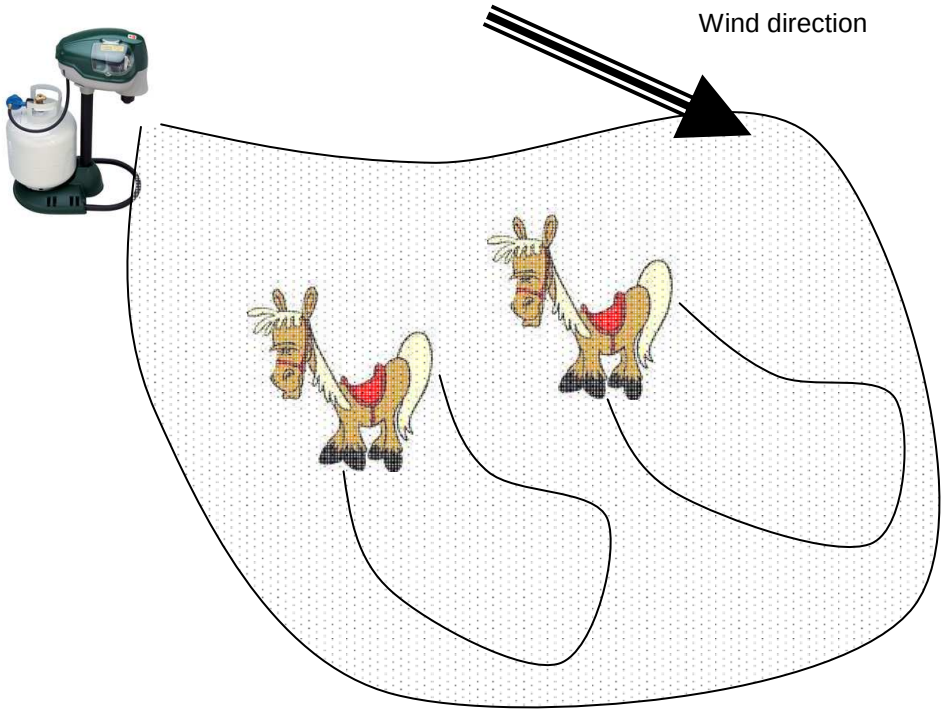

Figure 3.4 The plumes of attractive chemical stimuli that are used by blood seeking insects. The small chemical plumes created by the horses' daily respiration are eclipsed by the much larger chemical plume created by the upwind trap. This causes insects to bypass the animals and fly towards the trap (own diagram).

3.3 Materials and Methods

The site chosen for the trial was one where there had been cases of AHS for the past three years and was surrounded by other affected areas. It was the farm of Ms Jane Collier on the Dargledale farm road (GPS Ref: S 29°31'20.3"; E 30°15'23.8") located in the Midlands of KwaZulu Natal. There were 6 horses in the stable block which was situated about 200 metres away from the main farm residence and was in the middle of 4 differently sized paddocks. There were various types of habitat around the stable block including long kikuyu grass, low trees providing deep shade, wide open area of mowed grass and a large paddock of veld grass. Horses were stabled at night and kept in the adjacent paddocks during the day. They never

moved more than 200 metres from the stable block at any time during the experiment.

These traps are dependent on wind direction to be effective and should be placed upwind of the target area for best control (Mosquito Magnet, 2007). Upon discussion with the manufacturers, it was decided that the traps be placed on all four sides of the stable due to the variant wind direction experienced in KwaZulu-Natal. Sites were set up at twelve locations and numbered (Figure 3.5), and samples were taken at ten sites.

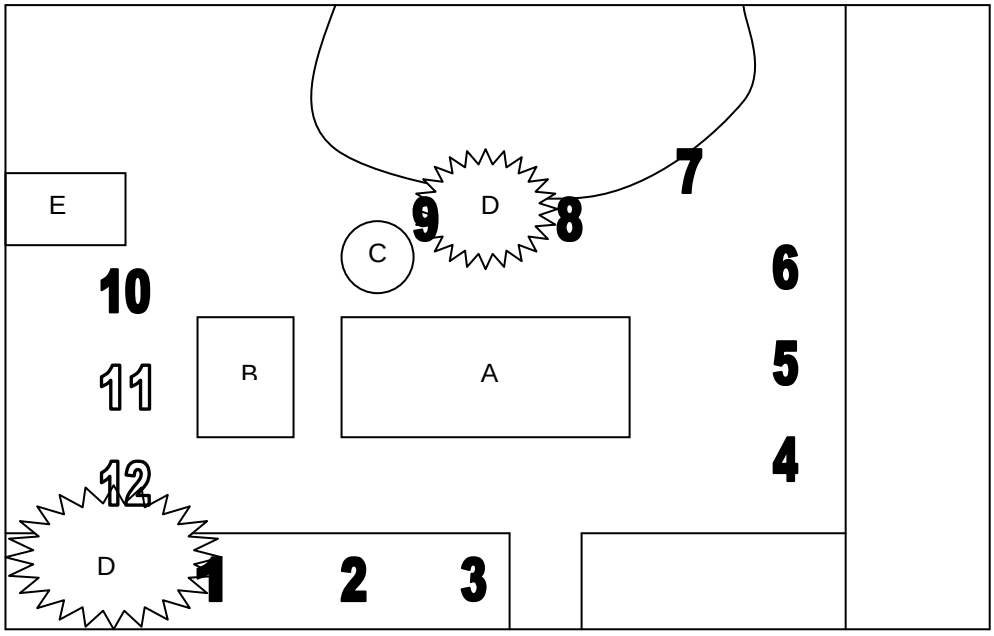

Figure 3.5 Arrangement of commercial traps around the stable block (A) containing 6 horses. Each trap was placed at each location for two consecutive nights. Other prominent structures were the tack shed (B), the water tank (C), the garage/storage shed (E) and two large trees (D).

The Mosquito Magnet Liberty Plus™ trap and the Mosquito Magnet Defender™ trap (hereafter called Liberty+ and Defender, respectively) both need an initial battery charge before being started, thereafter they use the power of the combusted propane gas. The ActiPower Trap ™ however, needs a constant 220V power source. It is only supplied with a 15 metre power cable so an extra 30 metre cable was added to it so that the trap could be used at all of the locations. Information from the Mosquito Magnet™ website explains that it takes 24-36 hours for the plume of attractants to establish in the environment downwind of the trap (Mosquito

Magnet, 2006). For this reason each site was sampled for 48 hours before being moved. At the end of the collection period, the traps were rotated within the three side positions first before being moved to the next side of the stable block. This allowed the collective plume from all three traps to remain in the area of the traps for a full 6 days before the traps were removed from that side of the stable. At the end of each two-day collection period, collected insects were placed into 85% alcohol, labeled and identified to taxonomic family or general group. Ceratopogonids were initially to be identified to species but upon inspection of the numbers caught, this was deemed unnecessary.

Catches were sorted and counted (Appendix 1). This individual class data was log (X+1) transformed (Holbrook and Bobian, 1989; Meiswinkel *et al.*, 2000) in an attempt to normalise the highly skewed data distribution. An ANOVA test was run on the log transformed data to discern significant differences between the numbers of insects caught in each class. To compare the efficacy of the three traps to one another, proportions of the total were calculated for each insect class and each trap. The significant differences given by a two-tailed Z-test indicated how efficiently each trap caught a certain type of insect as compared to one of the other traps. The *Culiciodes* midges were further identified to sex and stage of female reproductive development. The four stages identified were nulliparous, parous, gravid and blood fed. A two-tailed Z-test was used to provide an indication of how effectively each sex and female developmental stage was captured on a proportional basis by the traps.

3.4 Results and Discussion.

Catches were highly variable and consisted of three broad categories of insects; haematophagus flies, (being potential vectors of disease), non- haematophagus flies (of little veterinary importance) and other invertebrates. It can be seen from Table 3.1 that there was very high inherent variation in the magnitude of the catches for this style of trap. The haematophagus fly catches of the Liberty+ and Defender were 69.8% and 52% of the total catch, respectively, while blood sucking flies only comprised 35.15% of the total catch of the Actipower Trap.

Table 3.1 Numbers and groups of insects caught by three commercial insect traps over a period of twenty days in ten different locations at one farm. Mean percentages and standard deviations are shown below the total catch number.

	Defender		Liberty+		ActiPower Trap	
Total Insects	**348**		**221**		**308**	
Mean (**std dev**)	16.6	**35.9**	13.3	**16.5**	14.7	**26.4**
Total Haematophagus Diptera	**243**		**115**		**108**	
Mean (**std dev**)	40.5	**62.5**	28.8	**28.0**	18.0	**21.4**
Non Heamatophagus Diptera	**90**		**76**		**172**	
Mean (**std dev**)	9.0	**11.8**	10.9	**9.3**	24.6	**39.1**
Non Diptera	**15**		**30**		**28**	
% Heamatophagus Diptera	**70**		**52**		**35**	
	Haematophagus Diptera					
Midges (Ceratopogonidae)	**15**		**13**		**12**	
Mean (**std dev**)	2.6	**1.8**	1.9	**1.5**	3.0	**1.8**
Black Flies (Simulidae)	**166**		**70**		**59**	
Mean (**std dev**)	23.7	**20.4**	8.8	**6.5**	9.8	**14.6**
Mosquitoes (Culicidae)	**56**		**32**		**15**	
Mean (**std dev**)	5.1	**5.4**	3.6	**1.7**	3.0	**2.3**
Stable Flies (Muscidae)	**6**		**0**		**21**	
Mean (**std dev**)	2.0	**1.7**	0.0	**0.0**	7.0	**1.7**
Horse Flies (Tabanidae)	**0**		**0**		**1**	
Mean (**std dev**)	0.0	**0.0**	0.0	**0.0**	1.0	**0.0**

Of the many different taxa represented in each catch (Appendix 1) only the catches of the *Anopheles* spp. mosquitoes, moths and spiders returned any significant differences between traps ($p<0.05$) (Table 3.2).

Table 3.2 Log (x+1) transformed treatment means from an ANOVA test for the four groups of invertebrates that returned a significant difference ($p<0.05$) between catches.

Invertebrate group	p value	Defender Mean	Liberty+ Mean	ActiPower Trap Mean	s.e.	l.s.d.
Anopheles spp.	0.011*	0.496a	0.428a	0.060b	0.1362	0.2873
Moths	0.049*	0.000b	0.000b	0.108a	0.0476	0.1005
Parasitic wasps	0.007**	0.000b	0.220a	0.000b	0.0690	0.1455
Spiders	0.027*	0.060b	0.219a	0.060b	0.0612	0.1291

*=p<0.05; **=p< 0.01

Treatment means with different superscripts are significantly different.

s.e. = standard error

l.s.d = least significant difference

The traps are not designed to catch spiders or parasitic wasps. It is assumed that moths were caught in significantly higher numbers by the ActiPower Trap ($p<0.05$) because it is the only one of the three traps that has a light as well as the chemical attractants. This style of trap is designed to catch blood seeking insects and so an attempt to evaluate the efficacy of these traps was made. Due to the very low volumes of insects caught by each trap, an ANOVA test could not be effectively used. The proportion of the total catch contributed by each trap was calculated and compared using a 2-tailed Z-test. Table 3.3 shows the comparisons of each trap pair. The only group of blood sucking insects that showed any significant variation was the *Anopheles* spp. mosquitoes.

Table 3.3 Z-test values (critical value =1.96) showing the significance of catch proportions between traps for the various groups of haematophagus insects.

Trap Pair	Z-value	Taxonomic Group
Defender - Actipower	2.748**	*Anopheles* Mosquitos
Liberty+ - Actipower	3.225**	*Anopheles* Mosquitos
Liberty+ - Defender	-0.595	*Anopheles* Mosquitos
Defender - Actipower	0.547	Culicine Mozquitoes
Liberty+ - Actipower	-0.181	Culicine Mozquitoes
Liberty+ - Defender	-0.638	Culicine Mozquitoes
Defender - Actipower	1.434	Black Flies
Liberty+ - Actipower	0.424	Black Flies
Liberty+ - Defender	-1.305	Black Flies
Defender - Actipower	0.121	*Culicoides*
Liberty+ - Actipower	0.315	*Culicoides*
Liberty+ - Defender	0.188	*Culicoides*
Defender - Actipower	-1.281	Stable flies

** significant difference between trap catches.

No comparisons were made of the following data. Horse flies – only 1 was caught by the Actipower trap; stable flies – the Liberty+ did not catch any.

Table 3.3 shows that both the Defender and the Liberty+ traps caught significantly more *Anopheles* spp. mosquitoes than the Actipower trap (Figure 3.6). It should however be noted that even though the *Anopheles* mosquito is an important disease vector, a mean nightly catch of less than four insects is not ecologically significant, nor does it confer any level of efficacy to this style of trap in KwaZulu-Natal.

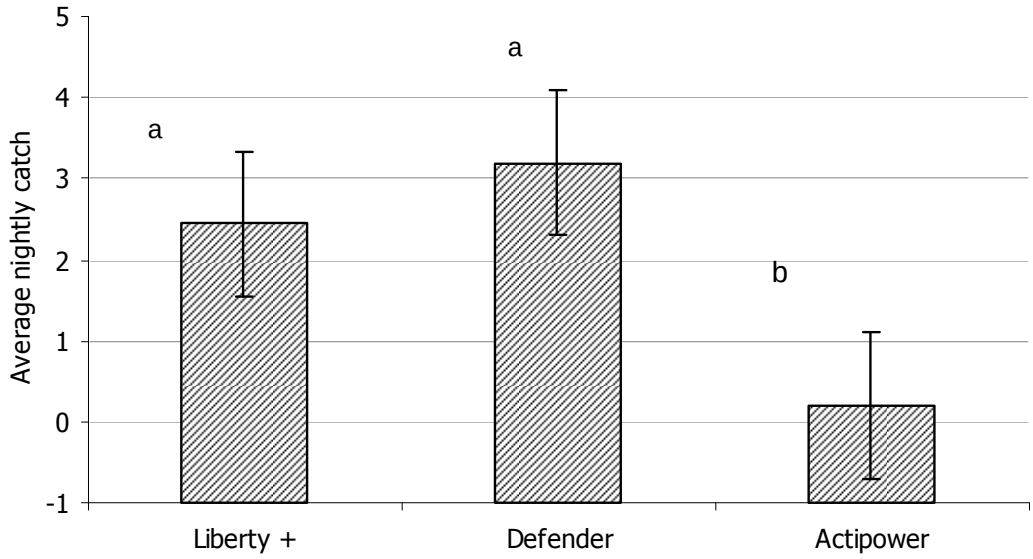

Figure 3.6 Mean nightly catches of *Anopheles* spp. mosquitoes across the tree different traps. Standard deviation is shown by the error bars. Colums with different superscripts differ significantly (Z test >1.96).

Midge catches were log (x+1) transformed in an effort to normalise the data. This transformed data was then run through a standard ANOVA. No significant difference could be assigned to either the trap ($p=0.909$) or the sex of the midges ($p=0.625$) but the "trap.sex" interaction showed significant differences ($p<0.01$). The sexes were preferentially attracted to specific individual traps. Table 3.4 shows both the transformed and back transformed nightly catches for the three traps.

Table 3.4 Log (X+1) transformed catch means showing significant differences in the trap.sex interaction.

Trap	***Sex***	***Log (X+1) Transformed Mean***	**Average catch**
Defender	Male	0.298 a	0.98
Liberty+	Female	0.264 a	0.83
Actipower	Female	0.186 ab	0.53
Actipower	Male	0.120 ab	0.32
Defender	Female	0.033 b	0.07
Liberty**+**	Male	0.000 b	0

Means with different superscripts are significantly different ($p<0.01$)

Table 3.4 shows that the Defender and the Liberty+ traps attracted significantly more males and female respectively. It is interesting to note that, even though the Actipower Trap has a UV light source, it did not out perform either of the other "pheromone only" traps. Its average catches for both males and females falls within the limits of both the other traps. It is not known why the Defender would attract more males as the combustion of the propane and the male pheromones are all intended to attract females. The Liberty+ however, was the only trap that did not catch any males during the trial period. For this reason, one could assume that the Liberty+ trap is more discerning in the out-trapping of *Culicoides* females. From a statistical standpoint, the Liberty+ trap is a wiser investment for the control of AHS vectors around a stable but, when the actual figures of insects caught is examined, a totally different story is told. None of the traps was capable of achieving a catch of just one midge per night! We know that midges were in the area during the period of the collection as emergence catches were made close to the date of this trial (see Chapter 4). Between all three traps over the 20 day collection period, only 40 *Culicoides* midges were caught.

These ranged across 12 separate species, the most numerous being *C. glabripennis,* of which 9 specimens were caught. This species breakdown of the summed total catches is presented in Table 3.5.

Table 3.5 Summed catch numbers of *Culicoides* spp. midges across all three traps over the whole collection period (20 days)

Culicoides Species	Total
C. bolitinos	3
C. brucei	1
C. expectator	1
C. glabripennis	9
C. glabripennis/ sp 75	2
C. gulbenkiani	5
C. imicola	5
C. krameri	1
C. magnus	7
C. nevilli	2
C. zuluensis	3
Unidentifiable *Culicoides*	1
Other Insects	437

Trap type, stage of female development and the interaction between the two was then run on a standard ANOVA. Neither the traps themselves nor the interaction showed any significant variation ($p=0.766$ and 0.819 respectively). However this style of propane combusting trap did catch highly significantly more nulliparous and parous females than gravid and blood fed females ($P<0.001$). Both stages may be attracted to the host animals with the aim of mating and thus being in a better position to mate and feed without having to cover any distance in between these two important acts. It is not known where these midges generally go to mate (if indeed there is a standard procedure from so variable a genus) but this may be one of the explanations. No blood fed females were caught by any of the traps. This is to be expected as they would not be attracted to host animals having just recently fed. Only one gravid female was caught.

3.5 Conclusions

The propane combusting style of trap has been shown to work very successfully at trapping mosquitoes in America (Takken & Kline, 1989; Kline *et al.*, 1994). The aim

of this trial was to determine their effectiveness as an out trapping aid for ceratopoginid flies of the genus *Culicoides* and, in this capacity, they are ineffective. With only small numbers caught over the full 20 day period, these traps cannot be said to be effective at out trapping large numbers *Culicoides* midges. This may be due to wind direction in KwaZulu-Natal being so variable (Jay, 2007; *pers.comm.* There may also be other explanations as to why they do not work for *Culicoides* midges. Possibly the 1-octen-3-ol is not potently attractive to this genus in general, or the genus might not be attracted strongly to the mixture of CO_2 and chemicals that have been shown to be attractive to mosquitoes. Whatever the reasons, for the capital outlay that is required to purchase these traps and maintain them in working order, they are not a suitable tool to control African Horse Sickness vectors in KwaZulu-Natal.

CHAPTER 4 : BREEDING SITES OF *CULICOIDES* MIDGES IN KWAZULU NATAL.

A. B. JENKINS[7] and G. J. VENTER[8]

4.1 Abstract

Locating breeding sites of disease vectors is an essential part of their control and the subsequent control of the diseases that they transmit. African Horse Sickness (AHS) is vectored by *Culicoides* midges and, while information is available on their breeding sites, not much data is available locally in KwaZulu-Natal (KZN). Following a period of design and trap trials, tent traps were made and placed at 90 locations in the KZN midlands. Catch numbers were correlated to site properties using the generalised linear modelling procedure on untransformed data with a negative binomial distribution and a log link function. Sites with increasing ground moisture, increasing incident radiation and increasing wetness duration were found to positively increase the number of midges collected from them. Possible applications of the results are discussed.

4.2 Introduction

Culicoides larvae have been found in a wide variety of habitats. Most often they occupy an ecotone where there is a wet substrate rather than free standing water (Mellor *et al.,* 2000). Many of the habitats from which *Culicoides* midges have been collected are outlined in Chapter One. Because of these highly diverse habitats, larvicidal control of midges is very difficult. If horse owners are aware of some habitat parameters in which *Culicoides imicola* breed, they will be able to take appropriate steps to suppress the population. For these sites to be known, understood and compared, midges must first be collected from a wide range of habitats. The numbers of midges caught at each site will then give an idea of where the vectors are breeding and will enable a larvicidal strategy to be implemented.

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The many methods of larval collection have produced a range of results. Braverman *et al.* (1974) used a simple flotation method whereby samples of possible habitat were added to salty water and the larvae simply floated to the surface. This worked well for many of the species except for *Culicoides imicola* which was shown by Nevill (1967) to sink when sampling by this method was attempted. The flotation technique was, therefore, disregarded for this trial as only data relating to AHS vectors, of which *C.imicola* is the most prevalent, was to be used. Lubega & Khamala, (1976) cut samples 6 cm deep and returned them to a temperature controlled environment where they were incubated for three weeks to allow all of the larvae to hatch. A similar method was used by Mellor & Pitzoltis, (1979) where a small hand trowel was used to collect samples only one inch deep and six inches in diameter. These samples were also artificially reared out for between three and four weeks. Randall (1982) used a spade to collect habitat samples in a bucket and then returned these to the lab. However, she did not have much success with this technique.

With emerging midges in the laboratory Bishop *et al.*, (1996) found that a temperature of between 25℃ and 28° achieved the mo st rapid emergence for *C. brevitarsis* and in a lighting experiment, Bishop *et al.* (1998) showed that constant light is also a stimulant for raising emergence numbers of *C. brevitarsis*.

The disadvantage of all of the 'removal' techniques is that the soil must be removed from the natural habitat and this is highly disturbing to the larvae, often killing them or stopping them from emerging altogether. When samples are removed to a laboratory, the conditions in the lab must also be as close to natural as possible. Plastic buckets with sealed lids do not work well as the samples are unable to dry out enough and promote the growth of fungi and bacteria. Only the emergence traps that are placed in the field are a suitable way of collecting midges from a habitat. Pajor (1987) designed a trap that is both robust, cheap and easy to construct. It was these traps that were used for the collection of the reported samples in this chapter. By using the most effective trap available, an understanding of the breeding habitats of AHS vectors can be obtained. These in turn can be targeted as possible sites for chemical application and larvicidal action.

4.3 Materials and Methods

4.3.1 Trap Design Process.

The design of the traps needed to collect samples from various larval sites was a process of trial and modification. Randall (1982) attempted to collect the larvae of *C. imicola* by collecting soil samples and trying to rear them out under artificial conditions. She had very little success and stated that a possible drawback to her larval sampling technique was that the samples were moved from their natural habitat and that this inhibited the emergence of the larvae contained therein. For this reason, collecting funnels were initially used for this trial. These consisted of a large plastic funnel from which issued a 20cm length of clear garden hosepipe (Figure 4.1). The hosepipe was doubled over itself and cable tied to the neck of the funnel. Attached to the end of it was a collecting vial filled with 85% alcohol. These very simple larval traps were pegged into the ground at various possible breeding locations found around the sites used for the adult collection trial described in Chapter 5. Upon inspection after a period of 2 weeks, they were totally unsuccessful and many were destroyed by inquisitive horses scraping them with their hooves.

Figure 4.1 Type 1 larval trap set up next to a pig effluent pond. Note the long top hosepipe is doubled back and attached to the side of the trap (own photograph).

Despite the experiences of Randall (1982) it was decided that samples would be cut with a spade and emergence would be done artificially. Collection of the soil samples was done very carefully so as to keep the soil structure intact as much as possible. Soil samples were cut from sites and placed in small 25cm diameter buckets. They were labelled and taken back to the laboratory (Figure 4.2).

Figure 4.2 Type 2 larval traps set up in the Entomology Department's laboratory (own photograph)

These were topped with the same funnels that were used in the field. The modification to the top of the traps was that the hose was shortened and bent through 180° so that the distance travelled by any midges would be reduced before they fell into the collecting alcohol. The samples were left on the bench top near a window so that close to natural conditions and temperatures would apply. A time span of one month was given to allow any insects in the ground to hatch and emerge. After 25 attempts using this method only a very small amount of insects were actually collected and, of these, there were no ceratopogonid midges. It was thought that the situation of the chambers in the laboratory was a detrimental factor as it was very cold at night and they were not exposed to direct sunshine during the day. In an attempt to solve the environmental problems, the collecting chambers were moved to an insectary where the temperature and lighting regimen could be carefully controlled (Figure 4.3). Bishop et al. (1996; 1998) showed that 25℃ – 28℃ and constant light were the optimal conditions by which to rear out adult *Culicoides brevitarsus* under artificial conditions.

Figure 4.3 Type 2 larval traps set up in the temperature and lighting controlled insectary. Traps were kept under constant lighting at 27℃ (own photograph).

Upon inspection of the soil samples after 2 weeks, many of them had gone very mouldy and many of the emerged insects did not enter the collecting vial but flew to the top bend of the hosepipe and then dropped back into the sample. It was thought that this was because of the repulsive odours emanating from the 85% alcohol in the vial. Therefore the alcohol was removed and each day, any samples there were collected in the dry vial were collected with an aspirator and placed into another vial of alcohol. After a month results of collected insects were still very low with only 3 midges being caught.

To enhance the ease of movement of the insects from the bucket to the vial, the hosepipe bend was removed and replaced with an inverted honey jar. This method made the collection of the samples easier and the catch numbers did rise. However 25 samples only yielded two midges. Samples may not have dried out sufficiently if they were kept in a sealed plastic bucket (G. J. Venter, 2007; *pers comm*).

Further discussion led to the manufacture of 23 traps according to the plan given by Pajor (1987). These are elegant, cheap and simple traps to construct and are highly efficient at sampling all of the insects in an area (Figure 4.4). They consist of a gauze tent supported by a central stake. Onto the top of this tent was a collecting bottle containing a 15% Savlon™ solution. The Savlon™ solution serves to keep the insects in very good condition for the duration that the trap stands out in the field. Pajor (1987) showed that catches remain fresh for up to 3 weeks using this type of trap. Modifications to the Pajor design included a smaller tent collection area of 75cm X 75cm and a tin can as the gauze support and for the collecting bottle stabiliser. These traps were not set up at the same farms as the adult collections.

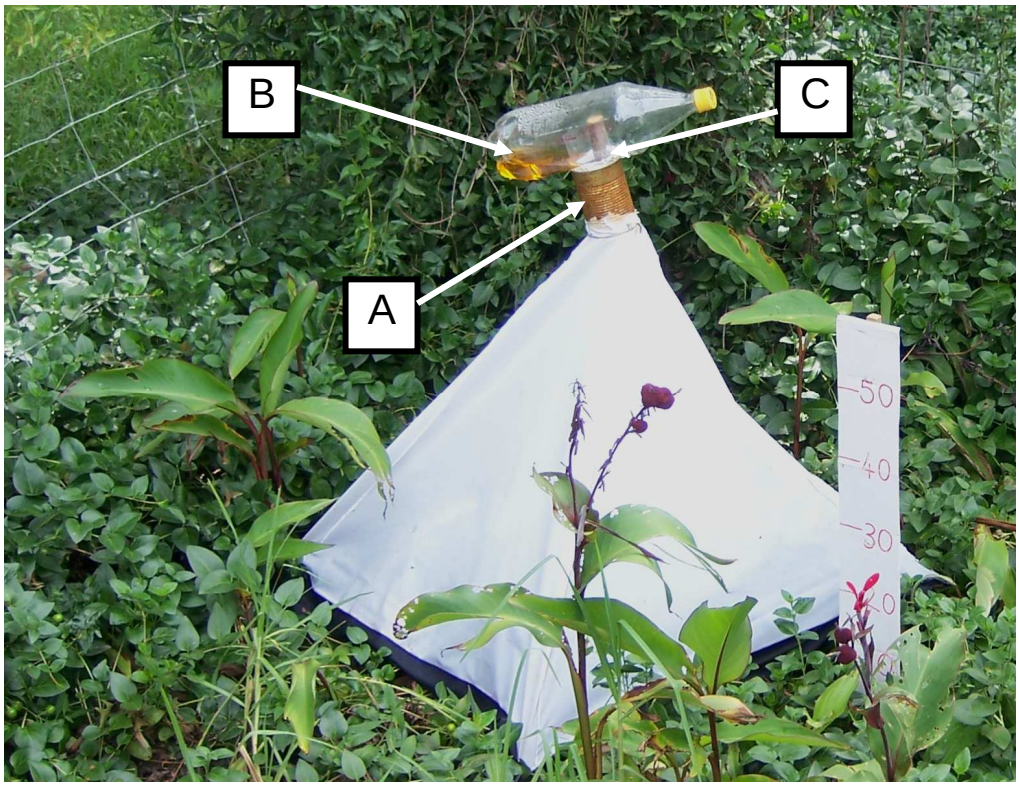

Figure 4.4 Emergence trap designed by Pajor (1987). Note A – Emergence chimney through which the insects enter the collecting bottle; B – 15% Savlon solution to capture the insects; C – Silicone sealant to keep the chimney joined tightly to the collecting bottle, and scale showing 10cm gradations on the right (own picture).

4.3.2 Collection Sites

Farms in Howick, KwaZulu-Natal, that had a history of the disease were chosen, and are given by their owner's names together with their grid references and elevations in Figure 4.5.

A) Taylor Farm	(GPS: S 29°31'38"; E 30°15'15")	A lt: 1213m
B) Burgouyne Farm	(GPS: S 29°31'58"; E 30°15'02")	Alt: 1130m
C) Fowler Farm	(GPS: S 29°30'47"; E 30°06'44")	A lt: 1133m
D) Helena Stewart Farm	(GPS: S 29°42'59"; E 30°18 '63")	Not recorded
E) Cedara Farm	(GPS: S 29°32'22"; E 30°16'02")	A lt: 1068m
F) Stewart Farm	(GPS: S 29°31'58"; E 30°15'02")	Alt: 1125m
G) Arnott Farm	(GPS: S 29°34'41"; E 30°13'31")	Alt: 1251m
H) Proctor Farm	(GPS: S 29°33'57"; E 30°14'08")	Alt: 1170m
I) Collier Farm	(GPS: S 29°31'20"; E 30°15'23")	Alt: 1065m
J) Hoskin Farm	(GPS: S 29°30'42"; E 30°15'17")	A lt: 1074m
K) Toucher Farm	(GPS: S 29°30'38"; E 30°08'22")	Alt: 1213m
L) Houston Farm	(GPS: S 29°31'28"; E 30°12'22")	A lt: 1088m

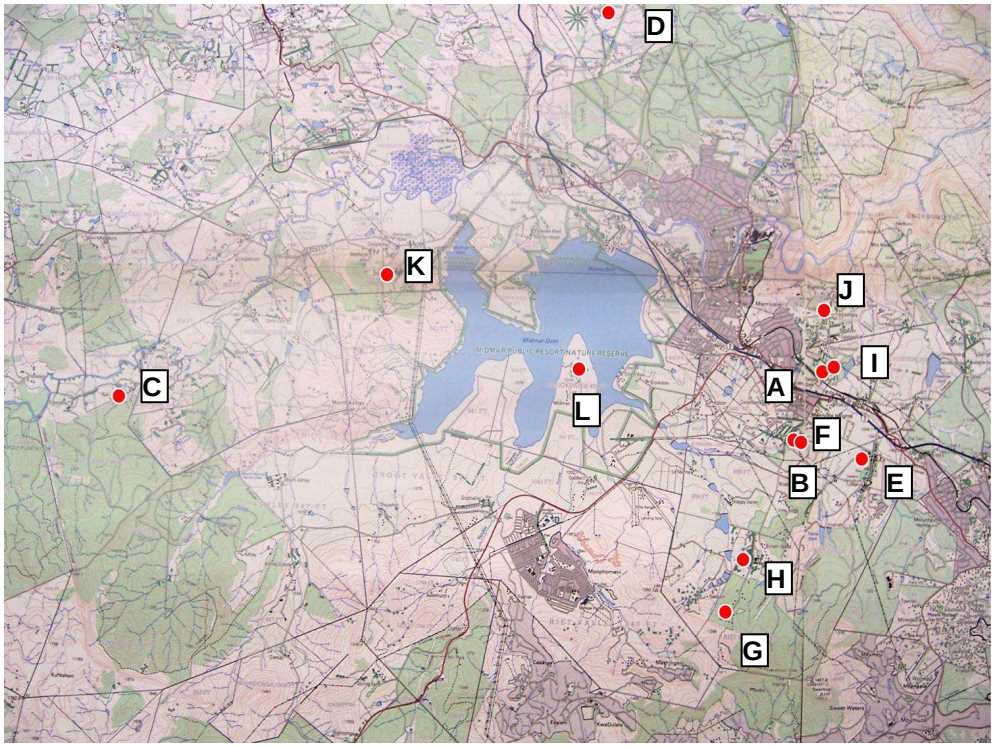

Figure 4.5 The locations of the 12 larval sites used as shown on a 1:50 000 scale map.

Traps were set up and the details of the site were divided into criteria that could be analysed statistically. These were;

Ground cover	kikuyu or another type of vegetation
Vegetation length	long (over 30cm) short (under 30cm) or none
Incident Radiation	sun, shade or deep shade
Moisture	dry, wet, or did it squelch under foot
Wetness Duration	permanently wet or temporarily wet by rains or irrigation

Pictures of each collection site and the grid references of the gates of each farm are given in Appendix 2. The traps were left on site for seven days and the catches were removed to 85% alcohol. They were sorted and only the Ceratopogonidae were retained. These were then sent to Dr. Gert Venter at Onderstepoort Veterinary Institute for identification to species and female maturity stage.

The resultant collections from the new traps were far greater than the collections made with the type 1 and 2 traps and it is believed that a representative sample of the habitat *in situ* was achieved.

4.3.3 Statistical procedure

The number of midges caught (total catch, males and females) was analysed using generalized linear modelling procedure in Genstat v9 2006©. The data followed a negative binomial distribution, as the data was non-normal count data where the relationship between the variance and the mean was not equal (McConway, 2006). A log link function was used. The significance of the parameter estimates in the accumulated analysis of deviance was used to establish the relevance of the treatments in affecting the total midge catch. Successful interventions in the control of AHS depend on the identification of the major parameters influencing total midge numbers around possible breeding sites. In this larval ecology study, the variation in response variates of total, male and females catch (using untransformed data) was attributed to levels of ground moisture content, permanence of ground wetness,

shade cover, ground cover and vegetation length at the trap site. Ground cover was confounded by vegetation length and so was dropped from the model.

4.4 Results

Total catches of all species across all of the collection sites are shown in Table 4.1. The most obvious trend is that the numbers are very low. Approximately 32% of midges caught were males and 68% were females. Table A3.1 in Appendix 3 shows how each collection site was related to the treatment factors. Estimates of parameters from the GLM procedure in Genstat are reported in Table 4.2.

Table 4.1 Species and total catch data of the female developmental stages and sexes of *Culucoides* midges caught by *in situ* larval emergence traps.

Culicoides Species	Nulliparous	Parous	Blood Fed	Gravid	Female	Male
C. bolitinos		3			3	1
C. glabripennis	1				1	
C. gulbenkiani		1		3	4	
C. imicola	4	1			5	1
C. leucostictus	30	5			35	18
C. magnus	5				5	2
C. neavei	1				1	
C. nevilli	1				1	
C. nivosus						1
C. pycnostictus	7	7		1	15	8
C. schultzei						1
C. trifasciellus						1
C. zuluensis	37	10		1	49	23
Total	86	27		5	118	56

Table 4.2 Parameters estimates of untransformed total midge numbers analysed by a negative binomial generalized linear model with dispersion parameter k=1 with a log link function in Genstat v9, indicating the response of ceratopoginid emergence in different breeding locations

Parameter	Estimate	se	T prob
Constant	-3.025	0.522	<0.001**
Moisture ◊	0.943	0.296	0.001**
Permanence of wetness ○	1.127	0.441	0.011*
Incident Radiation ♦	0.876	0.255	<0.001**

Levels of the treatment factors

◊ Moisture: dry (0) → wet (1) → squelchy (2)

○ Permanence of water wetness: temporarily wet (0)→ permanently wet (1)

♦ Incident Radiation: deep Shade (0) → dappled shade (1) → full sun (2)

An increase in the level of the factor was assumed to increase the probability of raising the catch of midges. For example an increase in substrate moisture is related to an increase in the total midge catch. In this manner, total midge catch is positively related to the ground water, with more midges emerging from soil that is wetter. Incident radiation increases the yield of midges, as does the duration of wetness of the emergence site (permanence). An increase in vegetation length was dropped from the model as it was not found to significantly increase total catch of midges.

Female midge catch numbers were positively increased by an increase in ground moisture and by an increase in the amount of incident radiation on the site. Table 4.3 shows the highly significant ($p<0.001$) relationship between the above two factors and the catch numbers of female midges.

Table 4.3 Parameter estimates of untransformed female midge numbers analysed by negative binomial generalized linear model with dispersion parameter k=1 with a log link function in Genstat v9, indicating the emergence of female midges from different breeding locations

Parameter	Estimate	Se	T prob
Constant	-3.956	0.689	<0.001**
Moisture ◊	1.226	0.260	<0.001**
Incident Radiation ♦	1.414	0.349	<0.001**

Levels of the treatment factors

◊ Moisture: dry (0) → wet (1) → squelchy (2)

♦ Incident Radiation: deep Shade (0) → dappled shade (1) → full sun (2)

Increases in male midge numbers were found to be highly correlated to increases in both ground moisture and wetness permanence (Table 4.4)

Table 4.4 Parameter estimates of untransformed male numbers analysed by negative binomial generalized linear model with dispersion parameter k=1 with a log link function in Genstat v9, indicating the emergence of male midges from different breeding locations

Parameter	Estimate	Se	T prob
Constant	-4.725	0.966	<0.001**
Moisture ◊	1.726	0.497	<0.001**
Permanence of wetness ○	1.930	0.787	0.014*

Levels of the treatment factors

◊ Moisture: dry (0) → wet (1) → squelchy (2)

○ Permanence of water wetness: temporarily wet (0)→ permanently wet (1)

4.5 Discussion

Culicoides bolitinos have only been found thus far in dung (Meiswinkel & Paweska, 2003). The findings of this trial showed C*.imicola* numbers were thinly spread across the few sites at which it was caught and *C. bolitinos* was found once in dung and twice under low trees in very deep shade (each incidence only yielding one midge). This issue has been experienced by other researchers in their attempts to discover the ideal breeding sites of AHS vectors (G. Venter, 2006; *pers. comm*) and remains a difficulty that will only really be overcome by a very wide ranging and long lasting trial involving collections over hundreds of possible breeding sites. The finding that 32% of midges caught were males is interesting when viewed together with the finding from Chapter 5 where only 8% of the adult midges caught were males. This demonstrates the possible longevity of female midges and the transience of the males.

The statistical model shows that midges are far more likely to emerge from very wet (squelchy) sites as opposed to drier ones. Dripping irrigation pipes that are moved frequently may possibly not be a concern. Such sites as dripping taps, gutter down-pipes, septic tank overflows, leaking reservoirs, edges of drainage ditches and small depressions that are always wet, are of greatest concern. Both male and female catch numbers were significantly increased by an increase in ground moisture and so reducing sites around the stables that are very wet and muddy will do much to lower midge populations.

Figure 4.6 demonstrates how a population of breeding midges can be dramatically reduced by simply removing the source of water that creates the boggy mud in which they breed. The reservoir was old and leaked from many places around its base. Collections were done near it in December and fairly large populations of midges were collected. By February, the farmer had decided that the threat of AHS was too great from this potential hotspot and so replaced the reservoir with a sealed plastic tanker. The catch in February was higher at all of the many sampled sites (details from chapter 5) but at this one site, was significantly reduced. This kind of

adjustment is highly effective at reducing midge numbers by removing their breeding sites.

Figure 4.6 Reservoir at a site in Karkloof and the catch of midges taken from there.

Wetness duration was highly correlated with increased catches of total midge numbers. This was also found for male midges but not for females. The most obvious reason for this correlation is that eggs need to be deposited so that the larvae can develop in a site that is not only suitable in the short term but at one that will remain optimal and ensure the survival of the young midges. The reason why permanently wet sites did not significantly alter female midge catches is unclear. Female midges may possibly have shorter developmental cycles when in their natural habitat but this has not yet been shown by any other researcher.

The increase in incident radiation on the breeding site was also interesting as breeding sites in the sun are far more likely to produce more midges than the same

site under shade. This trend is also true for the female midges and correlates well with findings on laboratory bred midges. Barnard (1998) found that more females were produced when eggs were incubated at higher temperatures. Whitman & Baylis (2000) also found that higher temperatures precluded more, smaller females than did lower temperatures. Having direct incident radiation on a site (sunny site as opposed to a site in shade) may well be the reason for there being more females from those sites.

4.6 Conclusions

The finding of the trial show various aspects of the reproductive cycle of *Culicoides* midges.

- breeding sites are small, widely spread and not uniformly chosen by any one species.
- Increases in moisture, permanence of wetness, and incident radiation all positively increase midge catches.
- Environmental modifications are highly effective at reducing midge populations at suitable breeding sites.

By modifying the environment where midges are most likely to breed, populations can be reduced and the potential viral challenge can be reduced in areas where AHS is a problem. Structural changes such as fixing leaking reservoirs and taps and adding concrete or sand to wet boggy areas may be useful in this regard. Larvicidal activity should be focussed on very wet sites that do not dry out and usually get a lot of sun if they are to be most effective. By vaccinating horses correctly and making some adjustments to stable infrastructure, a more comprehensive control of AHS vectors may be affected.

CHAPTER 5 : THE MOVEMENTS OF ADULT *CULICOIDES* MIDGES AROUND STABLES IN KWAZULU NATAL.

A. B. JENKINS[9] and G. J. VENTER[10]

5.1 Abstract

Preferences of adult *Culicoides* midges (Diptera: Ceratopogionidae) were examined to identify focal spots for vectors of African Horse Sickness (AHS). Five similar regions across five farms were sampled at regular periods over one year. The catches were identified to species level and regression analysis was performed on untransformed data which followed a negative binomial distribution with a log link function. Midges were found to frequent dung heaps and the interior of stable blocks significantly more than any other site ($p<0.001$). This occurs most markedly during July when temperatures are at their lowest and midges find shelter, warmth and food in these places. Recommendations for vector control with a suitable spray program are provided.

5.2 Introduction

Culicoides midges have long been known as the primary vector of African Horse Sickness (AHS) (Braverman & Chizov-Ginzburg, 1995). Devastating losses occur during epizootics of AHS (Barnard, 1998; Mellor & Hamblin, 2004). In South Africa where the virus is enzootic, losses are experienced every year. In the 2005/2006 season, 844 horses were positively identified with AHS and 148 of them died. This figure is possibly far below that actual numbers infected due to the system of disease confirmation that is in place in South Africa. The actual figures could be up to five times higher than those reported (Mullins, 2007; *pers. comm*.).[11]

Only two *Culicoides* midges have been proven as vectors of the AHS virus (Venter, 2007; *pers. comm.*). They are *C. imicola* (Du Toit, 1944), and *C. bolitinos*

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(Meiswinkel & Paweska, 2003). These two species are crepuscular (Meiswinkel *et al.*, 1994; Mellor *et al.*, 2000) showing peaks of activity over dusk and dawn. It is not known where the insects "roost" when they are not active, nor is it known where they are generally most active while they are mobile. Knowing the midge's preferred activity areas around a stable will greatly increase the efficiency of space sprays or fumigation techniques.

Midge numbers show a seasonal fluctuation with numbers dropping to their lowest during mid-winter and then rising again to peak in February and March (Coëtzer & Erasmus, 1994). A seven-year study showed a very significant increase in the midge population starting generally in December and peaking in March (Coëtzer & Erasmus,1994. This study was done at Onderstepoort and does not necessarily reflect the environmental conditions found in KwaZulu-Natal. The 2007 survey of AHS in South Africa showed KZN to be the second hardest hit region of the country after Gauteng (AHS website, 2007). It is therefore imperative that this data be collected from KZN so that it can be more effectively applied to the fight against AHS in the local setting.

It was the senior author's hypothesis that the adult midges would tend to aggregate in certain areas in or around the stable block. *C. bolitinos* had been shown to the endophyllic (i.e. prefer to enter stable blocks to feed) while *C. imicola* has been shown by to the exophyllic, preferring to stay outside and attack horses when they are not under cover (Meiswinkel *et al.,* 2000). Barnard (1997), however, found that *C. imicola* will actively enter stables to feed. The aim of this trial was to ascertain which of the two feeding styles is prevalent in KZN and to determine if there are any other behaviours particular to *Culicoides* species.

It is important to understand the movements of adult midges, over time, so that specific areas can be targeted for control. For example, if adult midges tend to congregate under environmental structures close to a stable block, this should be the site of any fumigation efforts. If an exophyllic species changes its behaviour during the cold evenings of winter, strategies should be adjusted accordingly.

5.3 Materials and Methods

Five stable yards across southern KwaZulu-Natal were chosen for sampling. Spurwing Farm in Karkloof (grid reference: 29°22'S; 30°14'E) has a large polo pony population and it has had a few cases of AHS in the past. It is situated in the Karkloof valley and the many horses are stabled from February to September and then kept in paddocks for the summer. There is a lot of agricultural land and the general countryside is high elevation grassland.

Cedara Agricultural College in Hilton (grid reference: 29°32'S; 30°16'E) has a small horse population of only six horses, but has experienced deaths in the past due to AHS. The stable block is closely surrounded by ploughed fields and the horses are kept inside a concrete stable at night and let out to graze freely during the day. The dung is kept in a large pile in front of the stable main door.

Ukulinga University Research Farm is 15km south west of Pietermaritzburg (grid reference: 29°40'S; 30°24'E) and was chosen because the seven large horses and nine miniature horses are kept in an open fronted stable next to the research feed lot where cattle, goats and sheep are kept. Accumulations of standing water abound. The stable block is surrounded by large paddocks on all sides. The dung is kept for the duration of winter in a large pile that runs the length of the stable block. In November this is removed and put on the paddocks and surrounding cultivated land.

Ashburton Training Center racing yards (grid reference: 29°38'S; 30°29'E) is approximately 11km southeast of Pietermaritzburg. There is a large population of race horses divided into individually managed trainer owned stable blocks. About 40 horses are kept in the cross shaped concrete stable blocks. The horses are kept indoors all of the time with the exception of early morning and late afternoon exercise periods and when they are racing. Dung is kept in a trailer which is removed when full approximately every five days.

Summerveld Racing and Equestrian Academy (grid reference: 29°50'S; 30°42'E) is about 30 km inland from Durban and is much closer to the coast than all of the other sites in this trial. It was chosen because of its coastal location, elevation and the fact that there has never been an instance of AHS at either the RAE or the racing yards or groom school that comprises the complex. There is a huge horse

population of over 300 kept in concrete stables. Dung is stored in large skips that are removed weekly.

At each of the farms, five sites were sampled. A description of these sites is given in Table 5.1. and site photographs are included in Appendix 4. All sites remained the same across the five sampling periods except for the annotated sites in Table 5.1. Sites were chosen so that there were roughly the same conditions across the five farms. That is, in an open expanse of grass well away from the horse' 'sleeping site: "Open Field' sites (shown in yellow), near the dung heap "Dung" sites (shown in purple), under the eves of the stable or inside the stable block "UnderIn" sites (shown in green), sites near an area of very boggy mud "boggy mud" sites (shown in grey), near the stables away from cover "near stables" sites (shown in red) and near open or standing water "open water" sites (shown in blue).

Table 5.1 Names of farms sampled and the sites at which two night light trap collections were made.

Farm Name	Site 1	Site 2	Site 3	Site 4	Site 5
Karkloof[c]	Next to large open pig effluent ponds	Reservoir overflow. Boggy Mud	In dung trailer. Removed weekly	Under eves in front of stables	[A]Open kikuyu paddock 50m behind stables
Cedara[c]	Sandy drainage ditch under long kikuyu grass	Open kikuyu field 80m from stable	Dry ground near concrete water trough	Top of dung pile in front of stable doors	Inside concrete stables. In empty stall
Ukulinga[c]	Small stream by compost ±500m from stables	Kikuyu field. At small concrete structure	Very boggy 40cm kikuyu by cattle feed lot.	Top of large Dung Pile in front of stables	Inside open stables. 3m under the eves
Ashburton	Inside half full 30Ton Dung skip	Inside X shaped closed stables. Center of stable	Boggy training ground and washing area	Open field under trees. 100m from stables	Outside stable near covered hay pile
Summerveld	Open paddock. Dry hard ground Short kikuyu	Boggy clay mud near through. No ground cover	Near covered stables under the eves	[B]Inside the Hay barn. Door always closed	Next to dung skip. Removed every week

A – Site was moved when the horses were let out for the summer so that it was always approximately 50m away from the horses.

B – Hay barn site was used twice and then changed to the middle of the stable block under a tree but not under the eves.

C - All horses kept in the stable block over winter but kept in the field from September till February.

Collections were taken over two consecutive nights at one farm before the traps were moved to the next. The traps were set up approximately two hours before sunset and ran until approximately two hours after sunrise. Sites were visited approximately every two months throughout the year on the following days;

Collection 1 -	2nd - 6th and 17th - 23rd of May 2006	following the first frost
Collection 2 -	17th – 27th July 2006	middle of winter
Collection 3 -	10th – 21st October 2006	beginning of high temperatures
Collection 4 -	4th – 15th December 2006	height of summer
Collection 5 -	12th – 23rd February 2007	height of the “midge season”

It was decided that a two-day sample would give a representative sample of midges for that period of the year and would serve to reduce the chance variation inherent in a single day’s sample. If the days sampled were extended to four or five days at each farm, the logistics of the project became prohibitively complex. This was because each night’s trap operation would run the battery almost flat, requiring it to be removed, returned to Pietermaritzburg and charged before being replaced in the traps the same afternoon. If sites were sampled for more than two days, travelling to all of the sites would take a significantly longer time to complete and this would then cause irregularities due to the changing weather patterns during the transition times of spring and autumn. A two day collection period was best with regards to the time spent collecting samples, and the time from the start to the end of a collection.

Sites were sampled using downdraught light traps equipped with an 8W blacklight as have been used in the past (Van Ark & Meiswinkel, 1992; Barnard, 1997; Rawlings *et al*., 1998; Musuka *et al*., 2001; Meiswinkel & Paweska, 2003; Rawlings *et al.*, 2003). As many different sites were to be sampled, the traps could not run on mains power and were instead modified to run on a 12V electric gate battery which needed re-charging after each night’s use. The traps were simple to build yet waterproof, sturdy, and highly effective as mobile collection tools. The basic design was two small, black plastic buckets mounted bottom to bottom either side of the downward facing blacklight housing (Figure 5.1). The top bucket was oriented so that the dorsally placed lid made access to the battery very easy. The battery was placed in the top bucket and attached with crocodile clips to the wiring of the trap. The bottom bucket housed the downdraught fan and was open at both ends to allow a free flow of air and specimens. The very fine grain collecting mesh funnel was attached to the bottom of the trap and a vial attached to the pinnacle of the funnel. The vial was filled with 70% alcohol to preserve all the insects caught. The whole trap was suspended from either a lashed tripod of thin gum poles or from the stable

structure. Using the tripod, the trap could be easily steadied on any terrain. The traps were set so that the blacklight bulb was approximately one and a half meters off the ground (Rawlings *et al.,* 2003). Finally upon setting up the traps, a standard mosquito net was placed around the entire trap to stop any large insects from entering the vial. It was found that this was effective at stopping all large insects from entering the trap therefore making sorting and identification easier.

Figure 5.1 Battery powered, blacklight, downdraught trap used to sample adult *Culicoides* midges. Note the alcohol filled vial at the bottom of the trap and the mosquito netting around the trap to filter out large insects such as moths.

The advantages of using this type of trap were mainly based around its portability. It could be set up anywhere and was very strong and stable. Due to the overhanging design, the collections withstood torrential rain and very strong winds. The weight of the battery suspended from the lashing on the top of the tripod, not only tightened the tripod joint but made the trap very hard to push over. It withstood the inquisitiveness of horses and dogs and got knocked over only four times in all 250 placements that were set. The trap was very portable and two traps could be

carried at the same time making setup fairly quick. The trap did not have to be near any type of power source and so was highly versatile in its ability to sample any location. The filling of the vial with alcohol helped a great deal as the insects were killed and thus could not escape if the trap somehow failed. Even when the trap got knocked over, the insects simply collected into the gauze funnel and were easily recovered the following morning. A disadvantage of the trap was that the battery had to be recharged every day. The total power usage of the trap was approximately 1Amp per hour and the gate batteries had a rating of 17Amp hours. Thus the traps could only function for 17 hours before the battery charge was depleted. This meant that the timing of the setting and removal of the traps was critical.

The number of midges caught was analysed using generalized linear modelling procedure in Genstat v9 2006©. The data followed a negative binomial distribution, as the data was non-normal count data where the relationship between the variance and the mean was not equal. A log link function was used (McConway *et al.,* 2006) to analyse catch data responses to location, species, sex, and reproductive status of female midges.

5.4 Results and Discussion

A total of 27 283 midges were caught, identified, sexed and aged. Figure 5.2 shows the total midge catches from each of the five locations across the year.

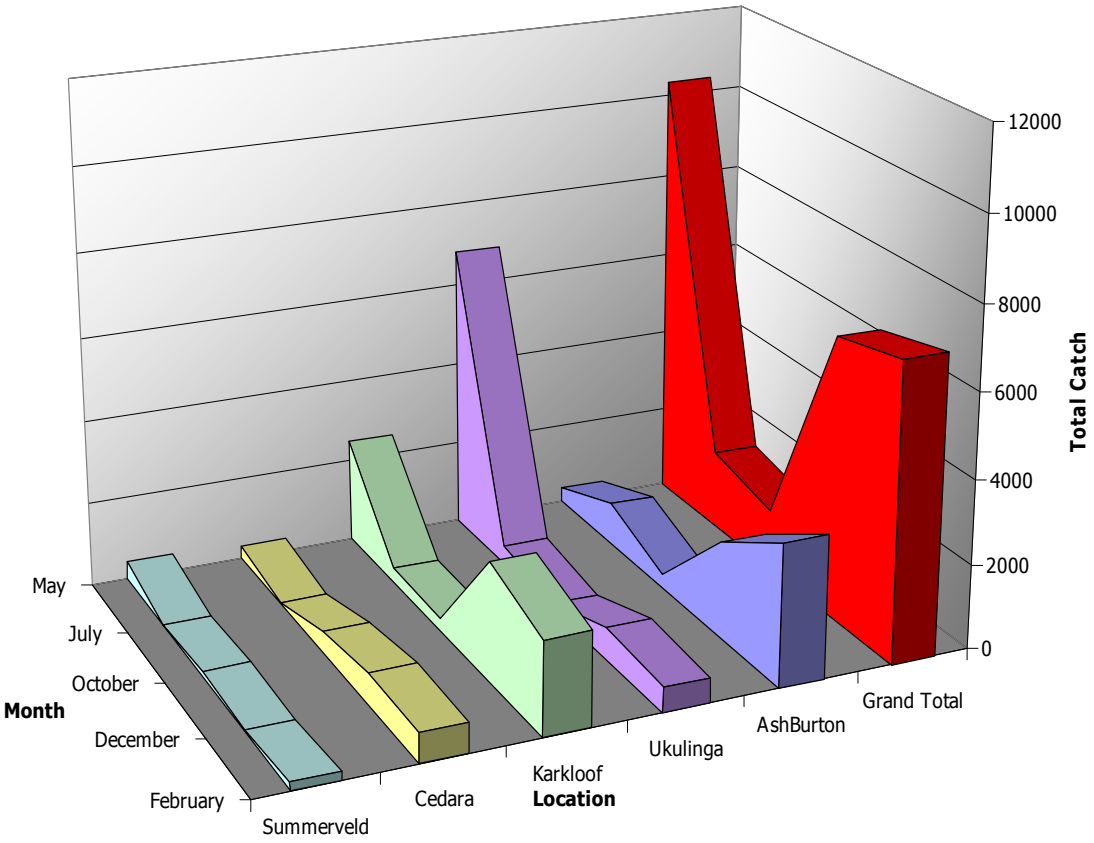

Figure 5.2 Total catches (grand total and by location) of *Culicoides* spp. across the trial period from May 2006 to February 2007.

A total of 37 *Culicoides* species were caught (Table 5.2). There are four species prevalent in KwaZulu-Natal, *C. imicola* (8903), *C. zuluensis* (6650), *C. bolitinos* (5660), *C. gulbenkiani* (2773). The other 33 species together produced 3307 individuals. These same four species were caught most often across all 250 collections: *C. zuluensis* (62%), *C. imicola* (60%), *C. bolitinos* (59%), and *C. gulbenkiani* (40%). The average catches and the largest single night's collection for the four species was: *C. imicola* (59 average and 938 largest single collection), *C. zuluensis* (43 average and 838 largest single collection, *C. bolitinos* (38 average and 828 largest single catch) and *C. zuluensis* (27 average and 659 largest single catch)

Across all catches, the percentage ratio of male midges to females 8%: 92%. This difference may be due to UV light preference by female midges, but more likely

points to a far greater population of female midges in the environment around stables.

Table 5.2 Species list and total numbers (males and females) caught across the year.

***Culicoides* spp.**	**Catch**	***Culicoides* spp.**	**Catch**
C. bedfordi	17	*C. neavei*	148
C. bolitinos	5660	*C. nevilli*	81
C. brucei	72	*C. nivosus*	260
C. coarctatus	4	*C. onderstepoortensis*	16
C. cornutus	2	*C. pycnostictus*	246
C. dutoiti	1	*C. ravus*	1
C. enderleini	133	*C. schultzei*	1
C. engubandei	675	*C. similis*	9
C. expectator	3	*C. sp. 107*	7
C. glabripennis	22	*C. sp. 51*	2
C. gulbenkiani	2733	*C. sp. 54*	56
C. huambensis	178	*C. sp. near angolensis*	5
C. imicola	8903	*C. subschultzei*	7
C. kibatiensis	21	*C. trifasciellus*	103
C. krameri	66	*C. tropicalis*	3
C. leucostictus	459	*C. tuttifruiti*	6
C. magnus	702	*C. unknown*	25
C. michelli	3	*C. zuluensis*	6650
C. milnei/kram	3		

For the purpose of disease control, both males and females are important. Males can be targeted for pheromonal control (Mordue & Luntz, 2003) and so reduce the breeding efficiency of the vector species. The females can be directly targeted at both their larval and adult stages. Most important of the female stages are the nulliparous (virgin females looking for their first blood meal) and parous (second incubation females looking for another blood meal) stages, as they both require an immediate blood meal and so are the mechanism by which the disease is vectored. As midges are small and not very robust creatures, the assumption is that not many will survive past their first egg batch to produce a second. By age grading of the females caught, it becomes apparent that the nulliparous females far outnumber all other stages (69.3% of all females caught). 23.2% of the females caught were parous, 7.3% were gravid and only 0.2% were blood fed). Venter *et al.*, (1996) suggested that the attraction of some *Culicoides* midges may change as they age or

experience different physiologic conditions. This theory would account for the very low numbers of bloodfed and gravid females caught.

5.4.1 Season

Across the year, a very obvious seasonal trend emerges. Populations rise during the hot months of summer and crash during winter. Figure 5.2 shows the seasonal variation in catches across the five locations and all follow a similar trend where the summer catch (December, February and May) are all generally higher ($p<0.001$) than the winter catches (July and October).

5.4.2 Location

Midge numbers were found to be significantly higher at Ashburton, Ukulinga and Karkloof than at Cedara and Summerveld ($p<0.001$). The high midge numbers at Ukulinga may have been a function of the very large dung pile that was located in front of the stables and in close proximity to both sheep and feedlot cattle. At Karkloof, the high numbers drop quite suddenly but then picked up again very quickly after the onset of summer. Karkloof is fairly misty and often wet. This and the stable's close proximity to a river may have been a factor allowing this marked increase in numbers from October to December. Horses at Karkloof are kept outdoors for most of the summer and this may be a reason why the midge catches during February are actually lower than those of December. Ashburton was the only site that showed an increase in midge numbers from the May 2006 collection to the July collection. The stable block in which the collection was made, was very tightly sealed, with paper stuffed in any possible entry points and all windows closed at all time. However, when the horses were being led in and out of the stable block, the doors were left open and were often found to be slightly ajar during the day to allow for some ventilation. The dung at Ashburton is pooled from two stable blocks and collected in a large 30 ton skip which is removed weekly. As the sides of the skip are very high, and the dung releases a lot of heat, a warm moist microclimate occurs within the skip and many different insects were noticed frequenting the interior of the skip. These two factors may well enable the midges to withstand severe cold periods during winter in areas where large concentrations of horses preclude a high

rate of dung production and good, warm stabling. Summerveld, while having a large supply of horses, does not have a significant midge population. This may be due to the location of the stables. They are situated on a fairly exposed bluff and often experience high winds and quickly changing temperatures. There is an almost constant wind that blows across the stable yards. Their dung is also cleaned out of the stables into a large skip which is removed weekly but there were not nearly as many insects in the skip as were at Ashburton. The Summerveld skip is not under any shade and so dries out quite quickly during the day. It is also smaller and so fills quickly. This may reduce the amount of available microhabitat within the skip. In contrast, the Ashburton skip is in deep shade, seldom gets full sun on it and is very large and so fills slowly. This allows an air column of warm stagnant air to form over the dung and may be a suitable microhabitat for all insects. Cedara was not found to have a significant midge population although AHS has been reported from the farm. The stable block is concrete and closed at night. Horses are kept out doors all day from dawn till dusk and the shut in at night. The site is not surrounded by trees and there is little standing water anywhere near the stables due to the very sandy soils. All of the above factors may conspire to make Cedara an unsuitable location for large midge populations.

5.4.3 Sites Within Each Location

The six sites sampled produced markedly different results. Table 5.3 highlights these where dung heaps and sites under eves or in stables produced significantly better catches than any other sites ($p<0.001$). Open field sites were significantly worse than all other sites at providing good midge catches ($p=0.005$). Sites near stables and those near open water showed no significant difference from the selected reference level and so are not dissimilar

Table 5.3 Parameter estimates, standard errors and t-probabilities of adult midge catches at six locations by generalized linear modelling procedure with a log link function (Genstat v9 2006©).

Site	Estimate	Std.error	T. Prob.
Dung Heaps [a]	0.695	0.133	<0.001**
Under In Stables [a]	0.515	0.134	<0.001**
Near Stables [b]	0.022	0.248	0.928
Boggy Mud [b]	reference level		
Open Water [b]	-0.204	0.173	0.240
Open Field [c]	-0.412	0.147	0.005*

Means with a different superscript differ significantly.

* P < 0.05 ** = P<0.01

Figure 5.3 shows the average nightly catches for male and female midges across all six selected collection sites. This diagram serves to accentuate the data given in Table 5.2 and shows the dramatic differences between male and female midge numbers.

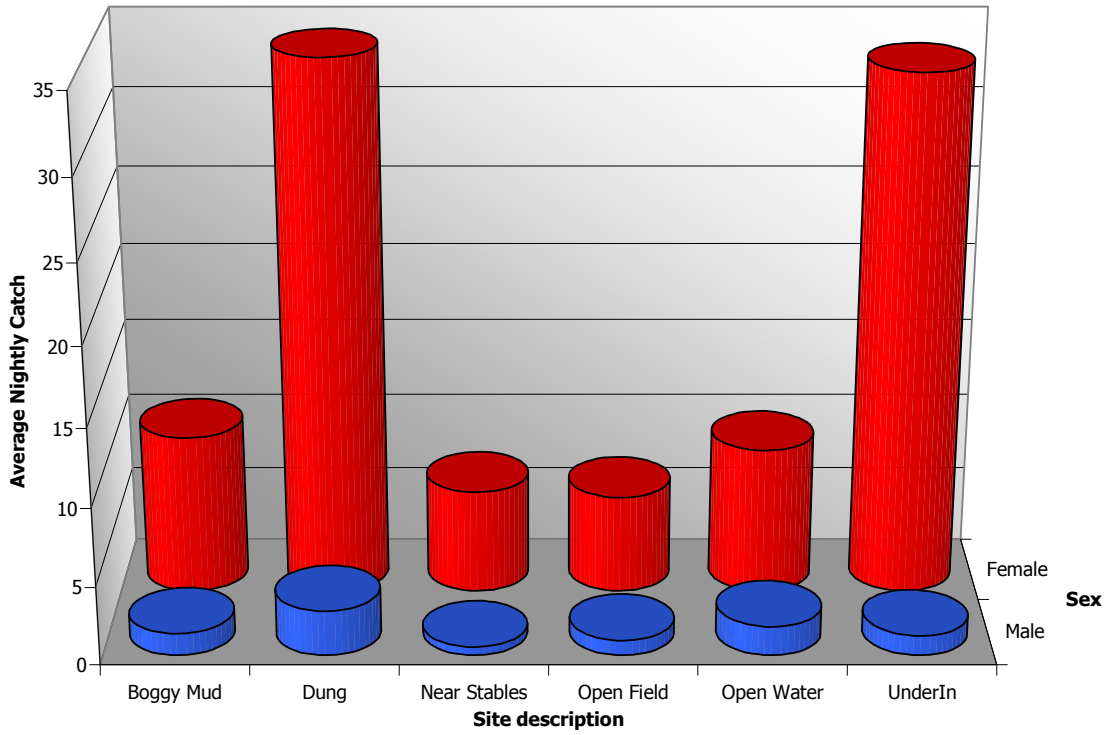

Figure 5.3 Average nightly catches for male and female midges across six selected collection sites located near stable blocks in KwaZulu-Natal.

The proportion of males and females at each site is not the same ($p<0.01$) (Table 5.4). Proportions of midges frequenting the dung sites are similar, but far more females entering the stable structure than males. This is to be expected as the females will mainly enter the stable to gain access to a blood meal. Male midges do not bite but suck nectar (Mullen, 2002) and so would not be attracted into a stable block.

Table 5.4 Percentages of total collections of males and female midges made at different selected sites in KwaZulu-Natal.

Site	Female %	Male %
Boggy Mud	8.9	15.3
Dung	41.9	44.1
Near Stables	1.4	1.3
Open Field	5.3	10.2
Open Water	4.4	10.9
Under In Stables	38.1	18.1

5.4.4 Species and sex differences across sites

Table 5.5 compares female numbers caught at each of the six collection sites. Across all species, the highest catches were from traps set on the dung piles, and then from sites located either under the eves or inside the stable. All other sites showed no significant difference from the selected reference level. This trend holds true at the species level as well.

Thus two hotspots emerge from the collected data; dung piles and inside the structure of stables. Both may be explained by the presence of a suitable microclimate created by factors in each place. On the dung piles, there is moisture, humidity, heat and a possible source of nutrients. Midges staying near the dung heap could be protected from wind by the barriers created by the structure of the dung. They would be buffered from environmental temperature changes by the heat emanating from the dung. This could give them opportunity to last the winter. If fresh, warm dung is continually added to the pile, the habitat would remain advantageous and may serve to extend a species longevity into winter. While it has been documented that female midges will drink honey when raised in the laboratory (Edwards, 1982) and may subsequently go on to produce up to nine batches of eggs, no observation has yet been made regarding their nutrient acquisition when they do not suck blood, although Lindley (1966) suggested that females may take in some type of carbohydrate source to help them survive until they are mature enough to have their first blood meal. Could the female midges derive nutrients from the dung between meals?

Table 5.5 Estimates, standard errors and t-probabilities of total female data as well as female data on the three main species collected across selected collection sites.

Site	Total females			*C. imicola* females			*C. bolitinos* females			*C. zuluensis* females		
	Estimate	s.e.	t. pr.	Estimate	s.e.	t. pr.	Estimate	s.e.	t. pr.	Estimate	s.e.	t. pr.
Dung Heap	2.611**	0.333	<0.001	3.485**	0.559	<0.001	2.214*	0.829	0.009	3.872**	0.745	<0.001
Near Stables	0.727	0.470	0.123	1.630	0.795	0.125	-1.430	1.390	0.308	1.600	1.010	0.115
Open Field	0.394	0.356	0.268	0.105	1.050	0.901	1.458	0.902	0.109	0.577	0.803	0.474
Open Water	-0.459	0.425	0.281	-0.905	0.844	0.357	-0.416	0.892	0.642	0.330	1.070	0.761
Under In Stables	2.076**	0.326	<0.001	2.395*	0.980	0.002	2.648**	0.773	<0.001	2.278*	0.714	0.002
Boggy Mud	Reference level against which all comparisons were made											

* = significantly different ** = highly significantly different

Large numbers of midges were also collected on or inside the stables. Again, a favourable microclimate is created that is free of wind, rain and extreme cold and can be exploited by the female midges during adverse environmental conditions. If horses are kept indoors all of the time, females are possibly willing to enter the stable block to acquire the all important blood meal. This is clearly demonstrated at Ashburton where very structurally sound stables house many horses. During winter, the stable interiors are warm and very comfortable while outside, night time temperatures dropped to zero degrees. The collections made during July at Ashburton produced no midges from the sites in the open field or near the stable block and only 3 midges from the boggy mud sites. However inside the stable block and the dung heap produced 116 and 891 midges respectively. A similar trend was noted across all sites whether the stable block was totally closed (Summerveld, Cedara, Ashburton and Karkloof), or open fronted (Ukulinga).

The migration of the majority of the midge population to dung heaps and inside stable blocks seems to only really occur during the depths of winter. Catches in July frequently turned up nothing at many of the collection sites, whereas collection in October were far better spread with many midges being caught at all of the sites.

5.4.5 Endophyllic or Exophyllic

Across all species, 37% of midges were caught either under the eves of or in the stables. Thus generally, midges are more exophyllic than endophyllic. But this is a large percentage which can not be ignored. 50.3% of *C. bolitinos* was caught in stables making it the most endophyllic of the main species caught. *C. imicola* and *C. zuluensis* both conformed to the overall average with 39.5% and 38% being caught indoors respectively. *C. gulbenkiani* was the most exophyllic of the main species with only 7.2% of specimens caught indoors.

5.5 Conclusions

These results can be put to good use in so far as vector control is concerned. By diligently cleaning out stables and removing dung throughout winter, the establishment of beneficial habitats can be reduced. A simple spray program can be used weekly in stable blocks to eradicate any midges that may have entered during the cold winter months. The data indicates that large scale impounding and concreting around the stable block would serve little purpose in removing midges from the vicinity of the horses on which they feed.

It would possibly reduce some breeding sites (refer to Chapter 4 for more on this) but the main focus should be:

- Remove dung as regularly as possible. If dung can not be moved more frequently than is currently done, consider a spray program to kill any midges that may be on the dung.
- Clean out and spray inside stables and under the eves in order to prevent any midges from using the shelter to survive winter.

CHAPTER 6: A COMPARISON OF *CULICOIDES* SPP. PREFERENCE TO LIGHT COLOUR AND SOURCE USING LIGHT EMITTING DIODES (LEDS) AND FLUORESCENT LIGHT

A. B. JENKINS

6.1 Abstract

Light colour and source preference testing has been conducted for *C. brevitarsus* in Australia but have not been performed in South Africa. Data from the Australian trials show an increased affinity for light form Light Emitting Diodes (LEDs) rather than incandescent light (the Australian Standard). Locally, the collection standard is an 8W fluorescent ultra-violet (UV) blacklight. A new trap was used to compare midge attractiveness to fluorescent and LED light sources as well as the colours: white, green and UV. Results show a very high affinity for UV light ($p<0.001$). Catches from white and green light were not found to be differ significantly ($p< 0.001$) and the interaction between light colour and source was not found to be significant. Possible trap development and action thresholds are discussed.

6.2 Introduction

Midges of the genus *Culicoides* have long been implicated in the transmission of many diseases afflicting both humans and livestock (Mellor *et al.,* 2000; Mullen, 2000; Wittman & Baylis, 2000) as outlined in Chapter 1. Effective study of this genus depends on the successful capture of the adult phase and an intimate knowledge of its life cycle and ecology. *C. imicola* and *C. bolitinos,* the two main species known to transmit African Horse Sickness have been described by many researchers as either crepuscular or nocturnal (Nevill, 1967; Walker, 1977; Boorman, 1993; Barnard, 1997; Wittman & Baylis 2000). It is this nocturnal or semi-nocturnal habit that is the key to catching midges. The initial, and very successful, use of light traps to catch *Culicoides* midges (Du Toit, 1944) sparked a lot of subsequent light trapping for research purposes. To date, the overwhelming trend in the collection of midges has been to use fluorescent black light as the main attractant (Walker, 1977; Van Ark & Meiswinkel, 1992; Venter & Meiswinkel, 1994; Barnard, 1997; Venter *et al.,* 2000; Musuka *et al.,* 2001; Meiswinkel & Paweska, 2003; Paweska *et al.,* 2003; Rawlings *et al.,* 2003; Labuschagne & Madjatladi 2005). Venter and Hermanides (2006) conducted a comparative study between black and white light as an attractant medium and found black light to be far superior to white. Some have

modified these traps to incorporate CO_2 as an added attractant (Holbrook & Bobian, 1989). Light traps have been modified to run off 12V batteries for added mobility (Rawlings *et al.,* 1998; Bishop *et al.,* 2000).

In a recent study on *C. brevitarsis* in Australia, different light colours and sources were tested for their attractiveness to midges Bishop *et al*. (2004). Results were compared to the catches of an incandescent bulb. It was found that there was a very significant increase in catch sizes when certain colours of Light Emitting Diodes (LEDs) were used to trap certain species of *Culicoides* midges. Green light emitted from LEDs at an intensity 142% that of an incandescent globe was almost three times more effective at trapping *C. brevitarsis* than the current collection systems used in Australia. They concluded that a new type of LED based trap would be better suited at sampling areas of sparse population as they are more attractive and so stand a better chance of drawing in a thinly spread population.

No comparative light-source work has yet been done in South Africa. LEDs are an excellent light source as they are more efficient than typical fluorescent or incandescent lights (G. Dewar; *pers. comm.*) and so any trap developed using LEDs can be run on batteries for a far longer period than would otherwise be possible. Any new improvements can be used in both the out-trapping of vectors from stables and the trapping of midges for research purposes. Light traps capture insects intact and often unharmed and therefore can be used to create a breeding population on which further studies can be made (Nevill, 1967). An increased knowledge of the conditions that attract midges will lead to a far superior research tool than is presently available.

A revolving LED and fluorescent light trap (Chapter 2) was used in the evaluation of light preference in the trapping of midges. Demonstrated light preference of AHS vectors can then be used to increase the effectiveness of light traps in controlling the spread of AHS through the control of the adult midge population.

6.3 Materials and Methods

A single revolving trap was set up at stables in Karkloof in the Midlands of KwaZulu-Natal (grid reference S29°22'47.9"; E30°14'43.3" GPS elev ation 1116m) during February 2007 when previous downdraught trap collections (using an 8W blacklight as the attractant) had yielded large catches of midges (Chapter 5). The light trap was turned on every evening an hour before dusk and was run until an hour after sunrise each morning. The trap was run for 15 consecutive nights which alternated between the two light sources. LEDs were run for eight nights and fluorescent lights were run for seven nights. Each morning, the trap was "cleared". Midges that had adhered to the Perspex© sheets were removed using No. 5 entomological tweezers. Samples were rinsed briefly in acetate to remove oil residues before being moved to 80% alcohol for preservation and storage. Midges were labelled and sent to Dr. Gert Venter at OVI for identification to family and genus.

Catch numbers were log (X+1) transformed to normalise the skewed distribution. Analysis of Variance (Genstat v9, 2006) of the transformed data was used to evaluate the interaction and main effects of type of light source and colour of light, with the expectation that the male and female midges would demonstrate a preference for coloured LED light.

6.4 Results and Discussion

The new revolving trap was very effective in catching midges (Figure 6.1). While the average nightly catch of 240 midges fell short of the 2243 nightly average for the 8W downdraught trap which was run a few weeks later at the same site, good data still resulted from the running of the new trap. The ANOVA demonstrates that UV LEDs would be the best way of catching midges. As these are very expensive, white LEDs would be the next best choice. 97% of the catch was female, of which 61% were nulliparous, 36% were parous, 0.1% were gravid and 2.9% were blood fed. Twenty different species were caught using the new trap.

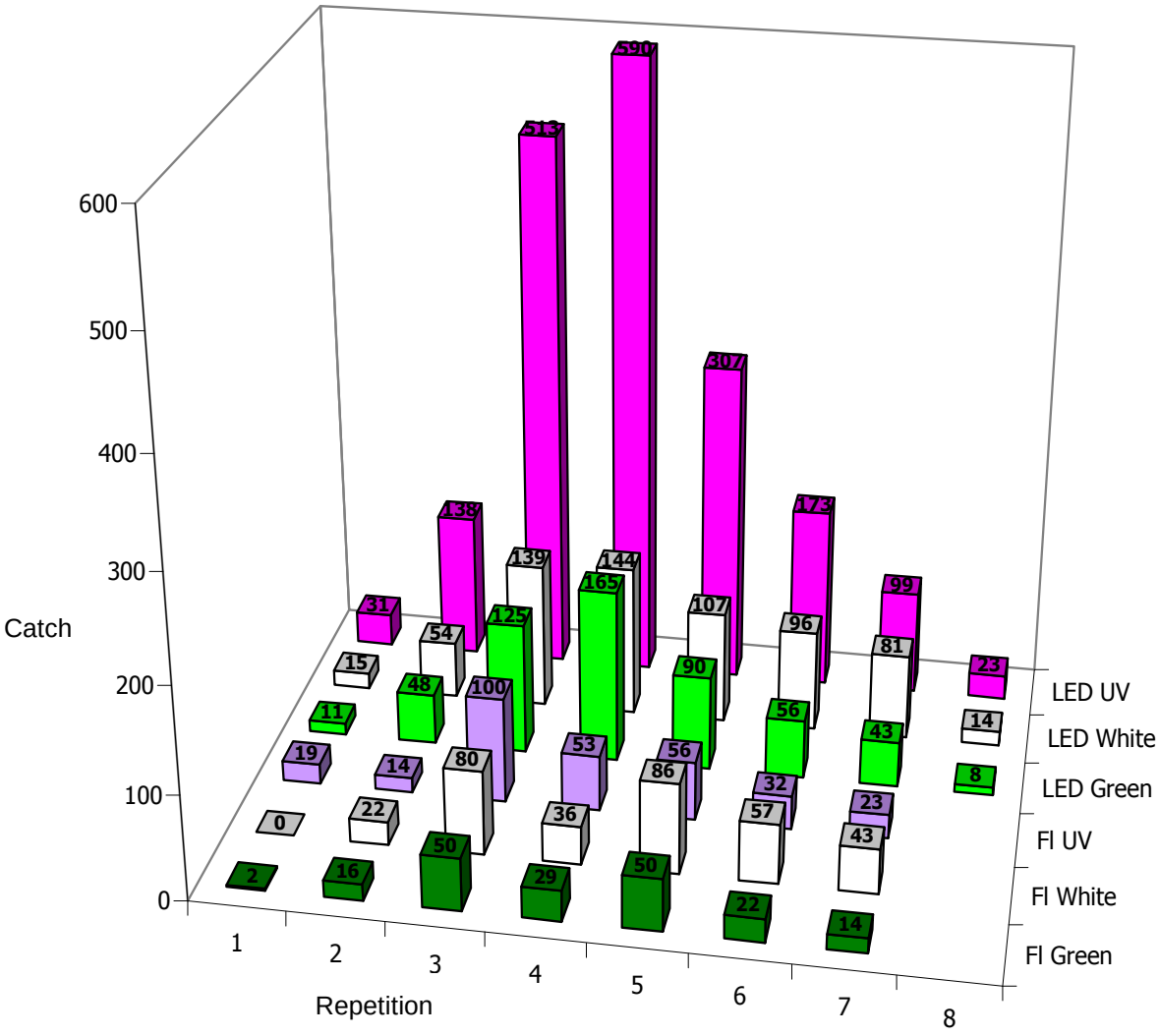

Figure 6.1: Catch numbers of *Culicoides* spp. male and female midges using six colour/light source combinations in a revolving light trap.

ANOVA showed highly significant variation between the different sources and colours of light ($p < 0.001$). However, the interaction between light colour and source was not significantly different ($p=0.865$). The log (x+1) transformed means shown in Table 6.1 highlight the usefulness of UV light as an attractant wavelength for midge collection. There is also significant difference ($p<0.001$) between LEDs and fluorescent lights as a light source. By percentage, this trend is seen across all species collected with all 20 species showing a higher percentage caught on the LEDs than on fluorescent lights. It was noted in the running of the trap that the light emitted by LEDs is far more directional and localised than that of fluorescent bulbs. When viewed from a distance, the fluorescent light did not appear to be as intense as the LED light. This is probably due to

the higher efficiency of the LEDs over the fluorescent bulbs. This directionality and intensity of light from the LEDs could attract insects from a wider area and may explain why the LED's would be more effective at attracting midges.

Table 6.1 Log (x+1) transformed means of *Culicoides* spp. using UV and coloured light combinations in a revolving light trap.

Colour ($p < 0.001$ l.s.d. 0.3403)			
UV	White	Green	Blank
1.874^{a}	1.636^{ab}	1.503^{b}	0.710^{c}

Light Source ($p < 0.001$ lsd 0.2412)	
Light Emitting Diode LED	Fluorescent
1.630^{a}	1.203^{b}

Means with different letters significantly differ from one another

l.s.d. least significant difference

UV light has been shown to be highly attractive to night flying insects and is an excellent bait for insect collections. It is therefore no surprise that UV light was the best colour of light with which to catch midges. Partially agreeing with the findings of Bishop *et al*. (2004), green light was not significantly different from white light but, in this instance, fell well below the attractive capacity of UV. The *Culicoides* genus is highly speciose and results pertaining to one specific species can not be extrapolated to another from a geographically different location. In all instances, the use of a light source was better than no light source at all. It is therefore important to turn lights off in the stables at night. The transformed mean catch from the LEDs is significantly higher than fluorescent light catches (Table 6.1), and the trap was effective in segregating the catches. This may be a very useful consideration in the design of small, cheap monitoring devices. LEDs are cheap, hardy and can be run on very low voltage systems. Thus the development of a battery or solar powered system may be possible through the use of LEDs.

6.5 Conclusions

With these findings, a few avenues of future research may be open. A practical tool can be developed for use by horse owners in order to empower them with the information necessary to make 'on-the-ground' decisions about their animals' safety. The technique

of an “action threshold” is currently enjoying widespread use in the control of Orange Wheat Blossom midge (*Sitodiplosis mosellana)* (Manitoba Agriculture, 2008). Small, yellow, sticky cards are set at various intervals throughout the crop. These attractive cards are checked periodically and when the critical number of insects caught within a period of time is exceeded, a spray regime is initiated to effectively control the pest population. Similarly, a battery operated LED panel with a removable sticky surface could be a good monitoring device for the control of *Culicoides* midges. A threshold number of midges caught per unit time can be calculated and this can initiate a control program involving larviciding possible breeding sites, fogging with an adulticide and an increase in pour-on prophylactics.

The revolving trap design, in testing light/colour combinations, also offers improvement value to the standard downdraught traps that are used in insect traps, by informing choices of light source and colour for geographically relevant control of particular midge species. In conjunction with a stringent vaccination program, the use of UV and LED light traps provides a practical intervention in the reduction of *Culicoides* midge populations to protect equids against African Horse Sickness.

CHAPTER 7: GENERAL CONCLUSIONS

Africa Horse Sickness remains a serious problem in South Africa, and identification of the species of midge vector, the larval and adult ecology as well as methods of trapping the adult and larval stages, are important to ameliorate interventions in control of the AHS virus. As an arbovirus, AHSV requires an arthropod vector for transmission from host and host. *Culicoides* midges, particularly *C. imicola* and *Culicoides bolitinos,* breed in many different habitats and, in the case of *Culicoides imicola* are capable of explosive population increases. Interventions for control of the adult and larval stages of the midge vector by identification of breeding sites and improved trapping procedures bodes well for improved control of the AHS virus vector.

In Chapters 2 and 6, a new trap was designed, described and used. The intention of this trap was to test the colour preference of AHS vectors with a view to suggesting the improvement of current collection apparatus with the most attractive light source and colour available. To this end it was extremely successful. While the trap is not capable of collecting large numbers of insects (in comparison with downdraught traps), it does inform the choice of light colour and source for midge trapping in downdraught traps in the South African context (Chapter 6). UItraviolet blacklight is the most attractive colour, but LEDs are superlative in terms of higher efficiency brightness in *Culicoides* sampling, and should therefore be incorporated into collection traps.

Chapter 3 compared three commercially available, propane combusting, downdraught traps, designed for mosquitoes, and indicated for use in midge control. These propane combusting traps were not effective at reducing the populations of biting midges around stable blocks in KZN.

Larval habitats were sampled from various farms across the KZN Midlands (Chapter 4), and midge yields were positively increased by an increase in site wetness, duration and incident radiation. Squelchy mud sites (that is, sites that may or may not have standing water but are very wet) that are permanently wet and subjected to a lot of sunlight are therefore the sites of which horse owners should take note. By applying correct quantities of larvicide or by changing the environmental factors that make the site permanently wet, horse owners can lower the chances of large midge populations breeding near their horses. This does not however totally prevent midges from breeding as midges were collected from all of the available site parameters. Horse owners should also be aware that midges, while fairly poor fliers, are capable of long distance dispersal in search of a

blood meal and so may attack horses away from their breeding sites. The most effective control of midges at their breeding sites is for groups of horse owners in an area to collectively decide to focus on breeding areas and therefore extend the range of larvicidal control. In this manner, effective larvicidal control could be realised and total midge numbers reduced.

Culicoides midges tend towards exophyllic activity with only *C. bolitinos* showing a preference for entering stables (Chapter 5). Midges congregate at dung heaps. The warm, humid microclimate makes dung heaps an ideal place for midges to rest when they are not sucking blood. Midges are very likely to be under the eaves of stables or in the stables themselves, where a warm, stagnant microclimate, shielded from rain and cold with an abundance of host.

Precautionary measures include frequent removal of dung heaps, turning dung heaps to dry them in the sun and reduce the humidity and warmth during winter or an insecticide spray application to kill any midges that are active there. By removing the midges from the dung heap, a large percentage of the population around the stables can be eradicated. Within the stables, interventions include fogging the stables when the horses are out for the day. Pour-on applications for horses when they are stabled at night as well as fans in the stables could be an effective way to deter midge activity (Simpkin; *pers. comm.*). Concerned horse owners do block up every small hole in their stables in an attempt to make them midge proof, but this unfortunately keeps the heat inside the stables and so acts as an increased attractant to midges, especially during the cold days and nights of winter. A suggestion is to keep stables well ventilated but with a fine gauze over all vents and windows. Midges are not caught in great abundance by boggy mud sites, where many larvae hatch out. This suggests that midges, when capable of flight, leave their larval habitats almost immediately in search of mates and food. Thus an adulticidal spray on possible breeding sites would not be as effective as a larvicidal application at the same locations.

By combining the findings that midges are most active at dung heaps and in stables and knowing that LEDs emitting ultraviolet light are most attractive, small cheap, portable traps with a series of sticky coverings on the front could be used to determine populations of midges at stable blocks. A threshold level of midges stuck to the trap over a predefined time can be correlated to bite load and therefore viral challenge. This can precipitate an

adult midge spray program, and also initiate the use of other interventions, as mentioned previously.

Investigations into the correlation between the bite load and viral challenge are being done at the University of KwaZulu-Natal (Young, 2007; *pers. comm.*). The degree of challenge at a biochemical level that will predispose a horse to the disease is important to know and is the subject of further study, and will hopefully also lead to the identification of the overwintering mechanism of the vector. Until that time, the convolution of circumstance that predisposes to a positive AHS event is being identified (Simpkin; pers comm.) and prophylactic strategies such as those suggested in this dissertation, as well as in the work of Simpkin (2007; unpubl.) should inform management practices in horse establishments. The effective control of larval and adult habitats, as well as the trapping and dissuasion of the vector are critical in the control of African Horse Sickness.

CHAPTER 8: REFERENCES

A.H.S. website, 2007 July. www.africanhorsesickness.co.za/map.

Actipower trap website, 2008 March. http://www.higieneambiental.com/control-de-plagas/actiupower-trap-trampa-de-exterior-para-mosquitos-1

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. Arch. Virol. 14, 13-19.

Baylis, M, Bouayoune, H., Touti, J., El Hasnaoui, H., 1998. Use of climatic data and satellite imagery to model the abundance of *Culicoides imicola*, the vector of African horse sickness virus, in Morocco. Medical and Veterinary Entomology. 12, 255-266.

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 (3), 202-205. (abstract)

Bishop, A.L., McKenzie, H.J., Barchia, I.M., Harris, A.M.,1996. Effect of temperature regimes on the development, survival and emergence of *Culicoides brevitarsis* Keiffer (Diptera: Ceratopogonidae) in bovine dung. Australian Journal of Entomology. 35, 361-368.

Bishop, A.L., McKenzie, H.J., Barchia, I.M., Spohr, L.J., 1998. Effects of lighting regimens on the emergence and numbers of *Culicoides brevitarsus* Keiffer (Diptera: Ceratopogonidae) in emergence chambers. Australian Journal of Entomology. 37, 319- 322.

Bishop, A.L., McKenzie, H.J., Barchia, I.M., Spohr, L.J., 2000. Moon Phase and other factors affecting light-trap catches of *Culicoides brevitarsis* Keiffer (Diptera: Ceratopogonidae). Australian Journal of Entomology. 39, 29-32.

Bishop, A.L., McKenzie, H.J., Spohr, L.J., Barchia, I.M., 2005. Interactions between dung beetles (Coleoptera: Scarabaeidae) and the arbovirus vector *Culicoides brevitarsus* Keiffer (Diptera: Ceratopogonidae). Australian Journal of Entomology. 44, 89-96.

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

Blackwell, A., 1997. Diel flight periodicity of the biting midge *Culicoides impunctatus* and the effects of meteorological conditions. Medical and Veterinary Entomology. 11, 361-367.

Blackwell, A., Evans, K.A., Strang, R.H.C., Cole, M., 2004. Toward development of neem-based repellants against the Scottish biting midge *Culicoides impunctatus*. Medical and Veterinary Entomology. 18, 449-452.

Boorman, J., 1993. Biting midges (Ceratopogonidae). In: Medical Insects and Arachnids. Eds. Lane, R.P., & Crosskey, R.W. Chapman and Hall, London, England. pp. 288-309

Bouayoune, H., Touti, J., El Hasnaoui, H., Baylis, M., Mellor, P.S. 1998. The *Culicoides* vectors of African horse sickness virus in Morocco: distribution and epidemiological implications. Archives of Virology Suppl. 14, 113-125. (abstract)

Braverman, Y., Galun, R. M., and Ziv, M., 1974. Breeding sites of some Culicoides species(Diptera: Ceratopogonidae) in Israel. Mosquito News. 34. pp.303-308.

Braverman, Y., Chizov-Ginzburg, A., 1995. Role of dogs (*Canis domesticus*) as hosts for African horse sickness virus. Veterinary Microbiology. 51, 19-25.

Bremer, C.W.,Gerdes, G.H., Aitchison, H., Louw, I. Et al. 2000. The prevalence of different African horsesickness virus serotypes in the Onderstepoort area near Pretoria, during an outbreak of African horsesickness in South Africa in 1995/1996. Onderstepoort Journal of Veterinary Research.

Capela, R., Purse, B.V., Pena, I., Wittman, E.J.,Margarita, Y., Capela, M., Romao, L., Mellor, P.S., Baylis, M., 2003. Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. Medical and Veterinary Entomology 17, 165-177.

Carpenter, S., Mordue (Luntz), A.J., Mordue, W., 2006. Oogenesis and laboratory survival in the Scottish biting midge *Culicoides impunctatus.* Physiological Entomology. 31, 89-93.

Coëtzer, J.A.W., Erasmus, B.J., 1994. African horse sickness. In: Coëtzer, J.A.W., Thomson, G.R., Tustin, R.C., Eds. Infectious diseases of livestock with special reference to southern Africa. Vol. 1. Oxford University Press, Cape Town, South Africa, pp. 460-475

Conte, A., Ippoliti, C., Calistri, P., Pelini, S., Savini, L., Goffredo, M., Meiswinkel, R., 2004. Towards the identification of potential infections sites for bluetongue in Italy : a spatial analysis approach based on the distribution of *Culicoides imicola*. Vet. Ital. 40, (3) 311-315.

Dallas, J.F., Cruickshank, R.H., Linton, Y.-M., Nolan, D.V., Patakakis, M., Braverman, Y., Capela, R., Capela, M., Pena, I., Meiswinkel, R., Ortega, M.D., Baylis, M., Mellor, P.S., Mordue (Luntz), A.J., 2003. Phylogenetic status and matrilineal structure of the biting midge, *Culicoides imicola,* in Portugal, Rhodes and Israel. Medical and Veterinary Entomology. 17, 379-387.

Du Toit, R.M., 1944. The transmission of blue-tongue and horse sickness by *Culicoides.* Onderstepoort J. of Vet. Sci. and Anim. Ind. 19. 7-16.

Edwards, P.B., 1982. Laboratory observations on the biology and life cycle of the Australian biting midge *Culicoides subimmaculatus* (Diptera: Ceratopogonidae) J. Med. Entomol.19, (5) 545-552.

E.L., 07/2006. Environmental Laboratory. Aquatic Plant Information System website. http://el.erdc.usace.army.mil/aqua/apis/biocontrol/html/overview.html

Fahrner, J., Barthelmess, C., 1988. Rearing of *Culicoides nubeculosus* (Diptera: Ceratopogonidae) by natural or artificial breeding in the laboratory. Veterinary Parasitology. 28, 307-313.

Friberg, C.A., Logas, D., 1999. Treatment of *Culicoides* hypersensitive horses with high dose n-3 fatty acids: a double-blinded crossover study. Veterinary Dermatology. 10, 117-122.

Gillies, M. T., 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bulletin of Entomological Research. 70. pp.525-532.

Gillies, M. T., and Wilkes, T. J., 1972. The range of attraction of animal baits and carbon dioxide for mosquitoes. Studies in a freshwater area of West Africa. Bulletin of Entomological Research. 61. pp.389-404.

Hamblin, C., Salt, J.S., Mellor, P.S., Graham, S.D., Smith, P.R., Wohlsein, P., 1998. Donkeys as reservoirs for African horse sickness virus. Archives of Virology Supplement 14. 37-47. (abstract)

Henning, M.W., 1932. African horse sickness, perdesiekte, *Pestis equorum*, in: Animal diseases in South Africa, Central News Agency , Limited, South Africa. pp. 516-538.

Holbrook, F.R., Bobian, R.J., 1989. Comparisons of Light traps for monitoring Adult *Culicoides variipennis* (Diptera: Ceratopogonidae). Journal of the American Mosquito Control Association. 5 (4), 558 – 562.

Hubers website, 2006 July. http://www.hubers.co.za/pests.html

I. N. R. A., 2006 July. National institute for Agricultural Research, France, Website http://www.inra.fr/Internet/Produits/HYPPZ/ZGLOSS/6g---022.htm

Johansen, C.A., Farrow, R.A., Morrisen, A., Foley, P., Bellis, G., Van den Hurk, A.F., Montgomery, B., MacKenzie, J.S., Ritchie, S.A., 2003. Collection of wind-borne heamatophagus insects in the Torres Strait, Australia. Australian Journal of Entomology. 17, 102-109.

Kettle, D.S., 1984. Arboviruses. In: Medical and Veterinary Entomology. Croom Helm Ltd, Kent, pp.451-474

Kettle, D.S., Edwards, P.B., Barnes, A., 1998. Factors affecting numbers of *Culicoides* in truck traps in coastal Queensland. Medical and Veterinary Entomology. 12, 367-377.

Kitaoka, S., 1982. Effects of rearing temperature on length of larval period and size of adults in *Culicoides arakawae* and *Culicoides maculates* (Diptera: Ceratopogonidae). Natl. Inst. Anim. Health Q.(Jpn.). 22, 159-162.

Kline, D.L., Hagan, D.V., Wood, J.R., 1994. *Culicoides* responses to l-octen-3-oll and carbon dioxide in salt marshes near Sea Island, Georgia, U.S.A. Med. Vet. Ento. 8. 25-30.

Lindley, J.R., 1966. The ovarian cycle in *Culicoides barbosai* Wirth & Blanton and *C.furens* (Poey) (Diptera: Ceratopogonidae). Bulletin of Entomological Research. 57, 1-17.

Linton, Y.M., Mordue (Luntz), A.J., Cruikshank, R.H., Meiswinkel, R., Mellor, P.S., Dallas, J.F., 2002. Phylogenetic analysis of the mitochondrial cytochrome oxidase subunit 1 gene of five species of the *Culicoides imicola* species complex. Medical and Veterinary Entomology. 16**,** 139-146.

Lord, C.C., Woolhouse, M.E.J.,Heesterbeek, J.A.P., Mellor, P.S., 1996. Vector-borne diseases and the basic reproduction number: a case study of African horse sickness. Medical and Veterinary Entomology. 10, 19-28.

Lord, C.C., Woolhouse, M.E.J., Mellor, P.S., 1997. Simulation studies of vaccination strategies in African horse sickness. Vaccine. 15 (5), 519-524.

Lubega, R., and Khamala, C.P.M., 1976. Larval habitats of common Culicoides Latreille (Diptera: Ceratopogonidea) in Kenya. Bulletin of Entomological Research. 66. pp.421-425.

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.

Minitoba Agriculture Website, September 2008, http://www.gov.mb.ca/agriculture/crops/insects/fad60s00.html

McConway, K. J.,Jones, M. C, Taylor, P. C., 2006. Further data analysis chapter 13, Statistical Modelling Using Genstat, Oxford University Press. New York.

Meiswinkel, R., 1989. Afrotropical Culicoides: A redescription of C.(Avaritia) imicola Keiffer, 1913 (diptera: Ceratopoginidae) with a 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. pp.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 imicola* (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. Bulletin of Entomological Research. 90, 509-515.

Meiswinkel, R., Nevill, E.M., Venter, G.J., 1994. Vectors: *Culicoides spp*. In: Coëtzer, J.A.W., Thomson, G.R., Tustin, R.C., Eds. Infectious diseases of livestock with special reference to southern Africa. Vol. 1. Oxford University Press, Cape Town, South Africa, pp. 68-89.

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

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

Mellor, P.S., Boorman, J., Baylis, M., 2000. *Culicoides* biting midges: Their role as arbovirus vectors. Annu. Rev. Entomol. 45, 307-340.

Mellor, P.S., Hamblin, C., 2004. African horse sickness. Vet. Res. 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. Bull. Ent. Res. 69, 229-234.

Mönnig, H.O., 1941. Arthropod parasites. In: Veterinary Helminthology and Entomology. 2nd ed. Bailliere, Tindall and Cox, Covent Garden, London. pp.263-387.

Mordue-Luntz, A.J., 2003. Arthropod semiochemicals: mosquitoes, midges and sealice. Biochemical Soc. Transactions. 31 (1), 128-133.

Mosquito Magnet, website. January 2007. www.mosquitomagnet.com.

Mullen, G.R., 2002. Biting midges (Ceratopogonidae). In: Medical and Veterinary Entomology. Eds. Mullen G., & Durden, L. Academic Press. London.

Murray, M.D., 1991. The seasonal abundance of female biting midges, *Culicoides brevitarsus* Keiffer (Diptera: Ceratopogonidae) , in coastal South-eastern Australia. Aust. J. Zool. 39, 333-342.

Musuka, G.N., Meiswinkel, R., Bayls, M., Kelly, P.J., Mellor, P.S., 2001. Prevalence of *Culicoides imicola* and other species (Diptera: Ceratopogonidae) at eight sites in Zimbabwe. Tydskr.S.Afr.Vet.Ver. 72 (2), 62-63.

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

O.B.P., 2004. Onderstepoort Biological Products leaflet P 2063 M. Control of African horse sickness. Edition 3.

O.I.E., 07/2006. Diseases Notifiable to the OIE. Office International des Epizooties. World organization for animal health. http://www.oie.int/eng/maladies/en_classification.htm

Ortega, M.D., Mellor, P.S., Rawlings, P., Pro, M.J., 1998. The seasonal and geographical distribution of *Culicoides imicola, C- pulcaris* group and *C-obseletus* group biting midges in central and southern Spain. Archives of Virology Suppl. 14, 85-91. (abstract)

Pajor, I.T.P., 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.

Paweska, J.T., Prinsloo, A.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. 17, 436-447.

Picker, M., Griffiths, C., Weaving, A., 2004. A field guide to insects of South Africa. Struik Publishers, Cape Town. South Africa. pp 270-271.

Randall, J.E., 1982. The distribution and ecology of *Culicoides* species (Diptera: Ceratopogonidae) at three sites in Natal. BSc Honours Thesis, University of KwaZulu-Natal, South Africa.

Rawlings, H., Capela, R., Pro, M.J., Ortega, M.D., Pena, I., Rubio, C., Gasca, A., Mellor, P.S., 1998. The relationship between climate and the distribution of *Culicoides imicola* in Iberia. Archives of Virology Suppl. 14. 93-102. (abstract)

Rawlings, P., Meiswinkel, R.M., Labuschange, K., Welton, N., Baylis, M., Mellor, P.S., 2003. The distribution and species characteristics of the *Culicoides* biting midge fauna of South Africa. Ecological Entomology. 28, 559-566.

Rawlings, P., Snow, W.F., Boorman, J., Denison, E., Hamblin, C., Mellor, P.S., 1998. *Culicoides* in relation to the transmission of African horse sickness virus in The Gambia. Medical and Veterinary Entomology. 12, 155-159.

Ritchie S.A., Harm, P., Van Essen, A., Kemme, J.A., Kay, B.H., Alaway, D., 1994. Response of biting midges (Diptera: Ceratopogonidae) to carbon dioxide, octenol, and light in southeastern Queensland, Australia. J. Med. Entomol. 31(5) 645-648.

Sæther, OA., 2000. Phylogeny of culicomorpha (Diptera). Systematic Entomology. 25, 223-234.

Scholtz, C.H., Holm, E., 1985. Insects of Southern Africa. Butterworths Publishers (Pty) Ltd. Cape Town. South Africa.

Service, M.W., 1971. Conservation and control of biting flies in temperate regions. Biological Conservation. 3 (2), 113-122.

Southwood, T.R.E., Henderson, P.A., 2000. Relative methods of population measurement and the derivation of absolute estimates. Ecological methods. Third edition. Blackwell Science Limited, Oxford, England.

Stram, Y., Brenner, J., Braverman, Y., Banet-Naoch, C., Kuznetzova, L., Ginni, M., 2004. Akabane virus in Israel: A new virus lineage. Virus Research. 104 (1), 93-97.(abstract).

Takken, W, Kline, D.L. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosquito. Control. Assoc. (3) 311-316.

Townley, P., Baker, K.P., Quinn, P.J., 1984. Preferential landing and engorging sites of *Culicoides* species landing on a horse in Ireland. Equine Vet. J. 16 (2), 117-120.

Unkles, S.E., Marriott, C., Kinghorn, J.R., Panter, C., Blackwell, A., 2004. Efficacy of the entomopathogenic fungus *Culicinomyces clavisporus* against larvae of the biting midge *Culicoides nubeculosus* (Diptera: Ceratopogonidae). Biocontrol Science and Technology. 14 (4), 397-401.

Vale, G. A., and Hall, D. R., 1985 (a). The role of 1-octen-3-ol, acteone and carbon dioxide in the attraction of tsetse flies (*Glossina* spp. (Diptera: Glossinidae), to ox odour. Bulletin of Entomological Research. 75. pp.209-217.

Vale, G. A., and Hall, D. R., 1985 (b). The use of 1-octen-3-ol, acetone and carbon dioxide to improve baits for tsetse files, *Glossina* spp. (Diptera: Glossinidae). Bulletin of Entomological Research. 75. 219-231.

Van Ark, H., 1975. The error involved in subsampling of large light trap catches of insects. Phytophylactica. 7, 1-8.

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

Venrick, E.L., 1971. The statistics of subsampling. Limnology and Oceanography. 16 (5), 811–818.

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 de K., 1996. Geographical distribution and relative abundance of stock associated *Culicoides* species (Diptera: Ceratopogonidae) in Southern Africa, in relation to their potential and viral vectors. Onderstepoort Journal of Veterinary Research. 63, 25-38.

Venter, G.J., Graham, S.D., Hamblin, C., 2000. African horse sickness epidemiology: vector competence of South African *Culicoides* species for virus serotypes 3, 5 and 8. Medical and Veterinary Entomology. 14, 245-250.

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.

Walker, A.R., 1977. Seasonal fluctuations of the *Culicoides* species (Diptera: Ceratopogonidae) in Kenya. Bull. Ent. Res. 67, 217–233.

Welby, M.P., Baylis, M., Rawlings, P., Mellor, P.S., 1996. Effect of temperature survival and rate of virogenesis of African horse sickness virus in *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. Bulletin of Entomological Research. 86 (6), 715-720. (abstract).

Whitman, E.J. Baylis, M., 2000. Climate change: Effects on *Culicoides*-transmitted viruses and implications for the UK. The Vet. Journal. 160, 107-117.

Whitman, E.J., Mellor, P.S., Baylis, M., 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis.* Medical and Veterinary Entomology. 16 (2),147-156.

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APPENDIX 1: RAW DATA FROM COMMERCIAL TRAP COMPARISONS

Table A2.1 Raw data from big Mosquito Magnet™ trap.

ORDER	DIPTERA																	OTHER INSECT ORDERS								
	Haematophagus flies																									
FAMILY	Ceratopogoinidae		Simulidae	Culicidae		Muscidae	Tabanidae	Drosophilidae	Cecidomyidae	Empididae	Sciaridae	Scatopsodae	Lauxiniidae	Tipulidae	Muscidae	Chironomidae	Psychodidae	Psochoptera	Staphilinidae	Beetles	Aphididae	Spiders	Moths	Fireflies	Formicidae	Parasitic Wasps
	Culicoides	Other		Culicinae	Anopheles	Stamoxys																				
Site 1			19		5				1		4						3	2								3
Site 2	2		12		2						4					7		1								
Site 3	1		10		2				2		1									1						1
Site 4	1		8		2				2				1	1												
Site 5	1		15								3			1		2		2	1		1	2				
Site 6	5		1		6				6		2						3	1				1				2
Site 7	1		4		2				1		1						1		1			2				
Site 8	2			4	3				4		3			1				2				2				3
Site 9			1	6				1	2		3			1			15		1			1				
Site 10 (#)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Total Numbers	13	0	70	10	22	0	0	1	18	0	21	0	1	4	0	9	22	8	3	1	1	8	0	0	0	9

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Table A2.2 Raw data from the small Mosquito Magnet™ trap

ORDER	DIPTERA																	OTHER INSECT ORDERS								
	Haematophagus flies																									
FAMILY	Ceratopogoinidae		Simulidae	Culicidae		Muscidae	Tabanidae	Drosophilidae	Cecidomyidae	Empididae	Sciaridae	Scatopsodae	Lauxiniidae	Tipulidae	Muscidae	Chironomidae	Psychodidae	Psochoptera	Staphilinidae	Beetles	Aphididae	Spiders	Moths	Fireflies	Formicidae	Parasitic Wasps
	Culicoides	Other		Culicinae	Anopheles	Stamoxys																				
Site 1	1		38		4									1						1						
Site 2	2		19		6				2							1	2									
Site 3	4	1	56		2	1		1	7		7						2	3		4						
Site 4	5		2		5	1			4	1	2		1		1		3			1						
Site 5			37		2															2		1				
Site 6	1		11	3		4			2					1					1							
Site 7					1			1	2			1					3									
Site 8		1		2	1				1								3				1	1				
Site 9			3		11						11			2		1	10									
Site 10				19					9		5						3									
Total Numbers	13	2	166	24	32	6	0	2	27	1	25	1	1	4	1	2	26	3	1	8	1	2	0	0	0	0

Table A2.3 Raw data from the ActiPower Trap™

ORDER	DIPTERA																	OTHER INSECT ORDERS								
	Haematophagus flies																									
FAMILY	Ceratopogoinidae		Simulidae	Culicidae		Muscidae	Tabanidae	Drosophilidae	Cecidomyidae	Empididae	Sciaridae	Scatopsodae	Lauxiniidae	Tipulidae	Muscidae	Chironomidae	Psychodidae	Psochoptera	Staphilinidae	Beetles	Aphididae	Spiders	Moths	Fireflies	Formicidae	Parasitic Wasps
	Culicoides	Other		Culicinae	Anopheles	Stamoxys																				
Site 1	2		1						1		1							2								
Site 2	5		39	5		8			72		8						18	12		2			1			
Site 3			3						1												1					
Site 4	4				1	5	1		9		5						4	3					1			
Site 5	1				1				1					1											1	
Site 6			9						2			1										1				
Site 7			5					1						1								1				
Site 8				6		8			19					3	3		3						2	1		
Site 9			2								5				1		1									
Site 10				2					5		3			1			2									
Total Numbers	12	0	59	13	2	21	1	1	110	0	22	1	0	6	4	0	28	17	0	2	1	2	4	1	1	0

APPENDIX 2: LARVAL SITE PHOTOGRAPHS

Figure A3.1 Site photographs of the 90 sites sampled for the larval ecosystem trial.

A). Taylor Farm sites (GPS: S 29°31'38"; E 30°15'1 5")

B). Burgouyne Farm sites (GPS: S 29°31'58"; E 30° 15'2")

C). Fowler Farm sites (GPS: S 29°30’47”; E 30°6’4 4”)






D). Helene Farm dung Site (GPS: S 29°42’59”; E 30° 18’63”)

E). Cedara Farm dung sites (GPS: S 29°32’22”; E 30 °16’2”)




F). Stewart Farm sites (GPS: S 29°31’58”; E 30°15’ 2”)




G). Arnott Farm sites (GPS: S 29°34’41”; E 30°13’3 1”)






H). Proctor Farm sites (GPS: S 29°33’57”; E 30°14’ 8”)







I). Collier Farm sites (GPS: S 29°31’20”; E 30°15’ 23”)





J). Hoskin Farm sites (GPS: S 29°30’42”; E 30°15’1 7”)

K). Toucher Farm sites (GPS: 29°30’38”; E 30°8’22”)

L). Houston Farm sites (GPS: S 29°31’28”; E 30°12’ 22”)

APPENDIX 3: LARVAL SITE DATA SHEET

Table A3.1 Physical properties of each sampled larval site. Number of collections and percentage of the total are given at the top of the table. The data was divided into sections and each site was given site scores. The higher the number, the more likely, that factor was of producing a midge breeding site.

% of Total Sample				**46**	**54**	**23**	**40**	**37**	**48**	**30**	**22**	**33**	**35**	**32**	**48**	**52**
Samples taken				**41**	**49**	**21**	**36**	**33**	**43**	**27**	**20**	**30**	**32**	**29**	**39**	**42**
#	**Farm**	**Description**	**Substrate**	**Ground Cover**		**Vegetation**			**Incident Radiation**			**Moisture**			**Wetness Duration**	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelchy	Perm	Temp
1	Liz Taylor	Household outflow into field	Kikuyu Mud	**1**			**1**		**2**					**0**	**1**	
2	Liz Taylor	Dog Kennel wash furrow through field	Kikuyu Mud	**1**		**2**			**2**					**0**	**1**	
3	Liz Taylor	Dog kennel wash boggy patch	Kikuyu Mud	**1**				**0**	**2**					**0**	**1**	
4	Liz Taylor	Dog kennel wash boggy patch	Kikuyu Mud	**1**				**0**	**2**					**0**	**1**	
5	Burgouyne	By top water tank at servants tap	Kikuyu Mud	**1**			**1**		**2**				**1**		**1**	
6	Burgouyne	Over drainage channel as it passes under road	Mud Stone		**0**			**0**		**1**			**1**			**0**
7	Burgouyne	Horse dung worm farm 44 gal drum with lid	Manure		**0**			**0**		**1**		**0**				
8	Burgouyne	Edge of stableyard under trees, compost	Leaf litter		**0**		**1**			**1**			**1**		**1**	
9	Burgouyne	Small hole in deep shade	Leaf Litter		**0**			**0**			**0**			**0**	**1**	

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
10	Burgouyne	Drainage ditch near path	Kikuyu Mud	**1**			**1**		**2**				**1**			**0**
11	Burgouyne	Dripping Tap behind building	Kikuyu Mud	**1**			**1**		**2**					**0**	**1**	
12	Fowler	In valley in the middle of hill side paddock	Kikuyu Mud	**1**		**2**			**2**				**1**			**0**
13	Fowler	Reservoir overflow. Very soft mud with grass.	Kikuyu Mud	**1**		**2**			**2**					**0**	**1**	
14	Fowler	Reservoir overflow watery mud no grass.	Open Mud		**0**			**0**	**2**					**0**	**1**	
15	Fowler	Marshy swamp amongst Arim Lillies	Reeds		**0**	**2**			**2**					**0**	**1**	
16	Fowler	Reservoir overflow. Flowing water and sparse long Veg	Velt grass		**0**	**2**			**2**					**0**	**1**	
17	Helena	Mixed Swedish Red dung. bulls, dry and dairy cows.	5 Cow Pats	**1**		**2**			**2**			**0**				
18	Cedara	Freisland Dairy cow dung	5 Cow Pats	**1**			**1**		**2**			**0**				
19	Cedara	Hereford dung. Low land by road. On Pasture	5 Cow Pats	**1**			**1**		**2**			**0**				
20	Cedara	Nguni cow dung. On pasture	5 Cow Pats	**1**			**1**		**2**			**0**				

	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
21	Cedara	Hereford dung. High land. On Pasture.	5 Cow Pats	**1**			**1**		**2**			**0**				
22	Stewart	By road to stables	Kikuyu mud	**1**			**1**			**1**		**0**				
23	Stewart	By Donkey trough on small aloe hill in deep shade	Weeds		**0**	**2**					**0**		**1**			**0**
24	Stewart	On a mowed kikuyu lawn in full sun	Kikuyu	**1**			**1**		**2**				**1**			**0**
25	Stewart	Corner of cow paddock in small rockey hole	Weeds		**0**	**2**				**1**			**1**		**1**	
26	Arnott	Under trees by road in flower bed. Deep shade	Mud		**0**			**0**			**0**		**1**		**1**	
27	Arnott	Kikuyu lawn that floods regularily	Kikuyu	**1**			**1**		**2**					**0**	**1**	
28	Arnott	Under hedge by protea nursery no light	Mud		**0**			**0**			**0**		**1**		**1**	
29	Arnott	Horse manure compost pile	Compost		**0**			**0**	**2**				**1**			**0**
30	Arnott	Under gum trees in shallow leaf litter	Gum leaves		**0**			**0**		**1**		**0**				
31	Arnott	By dripping tap next to riding field	Kikuyu	**1**		**2**				**1**			**1**			**0**
32	Proctor	Leaking Bowzer mud patch	Kikuyu	**1**			**1**			**1**				**0**	**1**	

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
33	Proctor	Behind stables by fence on sparse lawn kikuyu	Kikuyu	**1**			**1**		**2**			**0**				
34	Proctor	Under pine trees on the bed of needles	Pine Needles		**0**			**0**		**1**			**1**			**0**
35	Proctor	On Pine needles over muddy ground	Pine Needles		**0**			**0**		**1**				**0**	**1**	
135 **36**	Proctor	Under copse of black wattle trees	Wattle debris		**0**			**0**		**1**			**1**			**0**
37	Proctor	Under copse of black wattle trees	Wattle debris		**0**			**0**		**1**			**1**	**0**		**0**
38	Collier	In deep ditch dam covered with weeds.	Weeds		**0**	**2**			**2**					**0**		**0**
39	Collier	In deep ditch dam covered with weeds.	Weeds		**0**	**2**			**2**					**0**		**0**
40	Collier	Under trees on bare earth in paddock corner	Mud		**0**			**0**			**0**	**0**				**0**
41	Collier	Stable runoff ditch next to verandah	Kikuyu	**1**			**1**		**2**					**0**	**1**	
42	Collier	Behind stable under trees next to lung ring	Weeds		**0**		**1**				**0**	**0**				**0**
43	Liz Taylor	Squelchy mud Kikuyu Field	Kikuyu	**1**			**1**		**2**					**0**	**1**	
44	Liz Taylor	Squelchy mud Kikuyu Field	Kikuyu	**1**			**1**		**2**					**0**	**1**	
45	Liz Taylor	Wet Mud Kikuyu Field	Kikuyu	**1**			**1**		**2**				**1**		**1**	
46	Liz Taylor	Wet Mud Kikuyu Field	Kikuyu	**1**			**1**		**2**				**1**		**1**	

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
47	Liz Taylor	Dry Kikuyu Field	Kikuyu	**1**			**1**		**2**			**0**				**0**
48	Liz Taylor	Dry Kikuyu Field	Kikuyu	**1**			**1**		**2**			**0**				**0**
49	Hosking	Under low shade tree in bottom paddock.	Kikuyu	**1**			**1**				**0**	**0**				**0**
50	Hosking	Under low shade tree in bottom paddock.	Kikuyu	**1**			**1**				**0**	**0**				**0**
51	Hosking	Under low shade tree in bottom paddock.	Kikuyu	**1**			**1**				**0**	**0**				**0**
52	Hosking	Under jacaranda trees in bottom paddock	Kikuyu	**1**			**1**			**1**		**0**				**0**
53	Hosking	On Rubble Pile next to stables. Rocky ground	Weeds		**0**		**1**		**2**			**0**				**0**
54	Hosking	Near gateway to bottom paddock near dripping tap.	Sand		**0**			**0**		**1**			**1**		**1**	
55	Hosking	Behind stables over muddy drainage ditch	Mud		**0**			**0**		**1**			**1**			**0**
56	Hosking	Next to bottom stables over drainpit opening	Mud		**0**		**1**			**1**			**1**			**0**
57	Hosking	Under jacaranda trees over saw dust patch	Sawdust		**0**			**0**		**1**			**1**		**1**	
58	Hosking	Under jacaranda trees next to stable	Leaf litter		**0**			**0**		**1**		**0**				**0**

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
59	Hosking	Side paddock next to house. Long Kikuyu	Kikuyu	**1**		**2**				**1**		**0**				**0**
60	Hosking	Next to stand of pampas grass	Kikuyu	**1**			**1**			**1**			**1**			**0**
61	Hosking	Next to dripping tap over moss	Moss		**0**		**1**		**2**				**1**		**1**	
62	Hosking	Under Orange tree in front of house	Mud		**0**			**0**			**0**	**0**				**0**
63	Hosking	Next to pool in flower bed	Mud		**0**			**0**			**0**		**1**		**1**	
64	Hosking	Under flower bed of trees and bushes	Mud		**0**			**0**			**0**		**1**			**0**
65	Toucher	By House Under stairs next to stables. Open sludge	Mud		**0**			**0**		**1**				**0**	**1**	
66	Toucher	In front of house over small depression in Velt grass field	Velt grass		**0**	**2**			**2**				**1**		**1**	
67	Toucher	Amongst large hay bales under wattle trees	Hay		**0**			**0**			**0**		**1**			**0**
68	Toucher	Under wattle trees deep shade on sparse grass	Mud	**1**				**0**			**0**		**1**			**0**
69	Toucher	Between closely planted wattles	Wattle debris		**0**			**0**		**1**			**1**			**0**

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
70	Toucher	Over fallen wattle bark pile	Wattle bark		**0**			**0**		**1**		**0**				**0**
71	Toucher	Under fallen wattle tree over small mud patch	Mud		**0**			**0**		**1**				**0**		**0**
72	Toucher	In amongst a patch of Solanum Weed	Weeds		**0**	**2**			**2**			**0**				**0**
73	Toucher	Depression in velt Grass	Veld grass		**0**	**2**			**2**				**1**		**1**	
74	Toucher	In the road next to dripping tap	Kikuyu	**1**			**1**		**2**					**0**		**0**
75	Houston	Sunny flower bed by stables	Flowers		**0**	**2**			**2**				**1**		**1**	
76	Houston	Shady flower bed by stables	Flowers		**0**		**1**				**0**		**1**		**1**	
77	Houston	Deep shade and long grass behind stables by path	Kikuyu	**1**		**2**				**1**		**0**				**0**
78	Houston	Over stable run off over path behind stables	Kikuyu mud	**1**			**1**		**2**					**0**	**1**	
79	Houston	Under wattle trees over boggy kikuyu	Kikuyu	**1**		**2**					**0**			**0**	**1**	
80	Houston	On hay waste over squelchy ground	Hay waste		**0**			**0**	**2**					**0**	**1**	
81	Houston	Next to road at side of stables under pine trees	Weeds		**0**	**2**				**1**		**0**				**0**

#	Farm	Description	Substrate	Ground Cover		Veg Length			Position			Wettness			Wet	
				Kikuyu	Other	Long	Short	None	Sun	Shade	Deep	Dry	Wet	Squelch	Perm	Temp
82	Houston	In very long Kikuyu by shed no horse access	Kikuyu	**1**		**2**			**2**			**0**				**0**
83	Houston	Under bamboo top	Bamboo debris		**0**			**0**			**0**	**0**				**0**
84	Houston	Under bamboo bottom	Bamboo debris		**0**			**0**			**0**	**0**				**0**
85	Houston	In derelict hut amongst bug weeds	Mud		**0**	**2**					**0**			**0**	**1**	
86	Houston	Deep shade under trees in bottom paddock	Kikuyu	**1**			**1**			**1**		**0**				**0**
87	Houston	Over "dairy block" outflow next to horse manure pile	Manure mud		**0**			**0**	**2**					**0**	**1**	
88	Houston	Under scrap metal sheeting next to dairy	Mud		**0**			**0**			**0**	**0**				**0**
89	Houston	Next to leaking water towers clear water mud	Kikuyu	**1**			**1**		**2**					**0**	**1**	
90	Houston	Downhill from leaking tower over clover patch	Clover		**0**		**1**		**2**					**0**	**1**	

APPENDIX 4: ADULT TRAP SITE PHOTOGRAPHS

Figure A4.1 Site photographs of all of the adult midge collection sites.

Ashburton Sites

Site 1 : Removable dung skip near the stable blocks. Originally a 30 ton skip was used then it was replaced within a smaller skip with much of the dung in bags next to it.

Site 2 : Centre if a cross shaped stable containing approximately 40 horses.

Site 3 : near a very wet washing and lunging area. Note the permanent puddle in the field near the trap.

Site 4 : In the middle of a field approximately 100 meters away from any stables.

Site 5 : Near the stables but not under cover.

Cedara Sites

Site 1 : Boggy mud caused by runoff from a tractor wash. Graded at the February collection to make a concrete drain.

Site 2 : In an open field away from the stables but near the horses all day

Site 3 : Near a well built water trough that did not leak.

Site 4 : Next to the dung heap in front of the stables.

No photo was taken

Site 5 : Inside a sealed concrete stable block. All windows and doors closed at night.

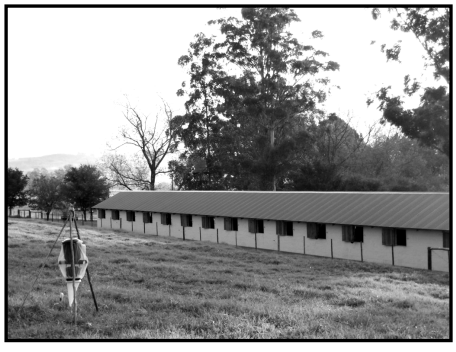
Karkloof Sites.

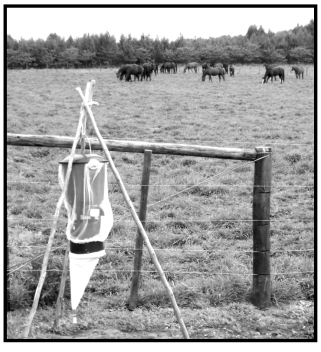
Site 1 : Next to three large pig effluent ponds.

Site 2 : next to a leaking reservoir. Note in the second picture, the reservoir has been replaced with a leak-proof Jojo tank causing the ground to significantly dry out.

Site 3 : Next to the dung trailers that were removed once a week. The large piles of wet hay and dung can be seen next to the trap.

Site 4 : In front of the stable block under the eves but not inside the stables.





Site 5 : Near the stables in a filed. Trap was moved close to horses during the summer collections as the stables were not used at all.

Summerveld Sites

No photo was taken

Site 1 : Trap in an open field. A very exposed site that was often windy.

Site 2 : Next to a permanently muddy area created by a leaking tap and water trough.

Site 3 : Under the eves of the stable block.

Site 4 : Next to the stables. Under a tree in the centre of three stable blocks.

Site 5 : Next to the dung skip that was removed weekly.

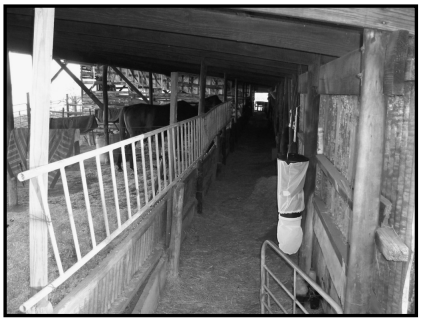
Ukulinga University Research Farm Sites

Site 1 : Next to a small very slow flowing river at the bottom of the farm. About 200m away from the stables.

Site 2 : Out in the open field next to a small disused pump house.

Site 3 : Near the stables on top of the marshy kikuyu created by the stable and r\feedlot run off. Very long kikuyu ground cover.

Site 4 : On top of the very large dung heap that is only cleared annually in front of the open fronted stables.



Site 5 : At the back of the open fronted stables. Well away from the wind and rain.