THE OCCURRENCE OF *Nosema apis* (ZANDER), *Acarapis woodi* (RENNIE), AND THE CAPE PROBLEM BEE IN THE SUMMER RAINFALL REGION OF SOUTH AFRICA

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ABSTRACT

The occurrence of Nosema disease, tracheal mites and the “pseudo-parasitic” behaviour of Cape honeybee workers when placed amongst African honeybees – known as the Cape Bee Problem – were studied over a 18 month period. Three surveys, approximately 6 months apart were done. The aims of this study were to establish the distribution and severity of the diseases and compare the disease with the presence of the Cape Bee Problem.

Before this survey commenced European Foul Brood disease, Sacbrood (virus), Nosema, Brood nosema, and Tracheal mite have sporadically been reported in the summer rainfall region of South Africa.

In the first survey 1005 colonies in 61 apiaries were surveyed, 803 colonies in 57 apiaries in the second, and 458 colonies in 41 apiaries in the third. Samples for disease and parasite analysis were taken at 4 colonies per apiary. Ten colonies per apiary were inspected for Cape Problem Bees, and samples of workers were collected and dissected at each of these colonies.

Even with the addition of apiaries to 'fill-up' lost colonies during the second survey, 63% of all colonies were lost by the third survey. There was only a small difference in colony loss between sedentary and migratory beekeepers of 22% compared to 27%.

Nosema was more prevalent amongst commercial beekeepers and increased in migratory operations during the survey period. The percentage of colonies infected increased during the survey period from 23% to 32% to 34%. The placement of colonies in Eucalyptus plantations may boost infection.

Trachea mites seem to have spread quite rapidly in South Africa since its discovery. This parasitic mite was present in all regions, although in low numbers in three most northern regions. Sedentary colonies had higher levels of infestation than migratory colonies. The number of colonies infested diminished over the survey period, which may be a result of general colony loss.

The Cape Problem Bee was less of a problem than anticipated. Colonies succumbed to Cape Problem Bees in all regions. When beekeepers reported high levels of infestation in their bee stocks the colonies would be dead within six months. In apiaries with low infestation the die-out was slower.

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INTRODUCTION

By studying fossil records, Culliney, (1983) estimated that honeybees evolved about 35 million years ago. This is much earlier then the estimated time that *Homo erectus* evolved, 800 000 to 500 000 years ago (Dobzhansky, 1962). It took millennia before humans evolved enough to convey their everyday life in a lasting way. The oldest surviving records are carvings and rock paintings, and it is in these paintings that the first association between man and bee is depicted. It can be argued, with good reason, that man ate honey and bee-brood long before they started to depict their association with the bees. The earliest undisputed evidence of humans 'robbing' honeybee nests is from a rock painting in eastern Spain and has been provisionally dated to the period 8000 - 2000 BC. However, there are earlier rock paintings dated at about 30 000 - 9 000 BC that arguably show honey being robbed (Crane, 1983). This is evidence that even these early humans regarded honey, and probably pollen and brood, as food.

The earliest evidence of beekeeping, that is keeping bees in artificial nests (called hives), probably arose with other farming methods as signified by the drawings from Egypt of around 2400 BC (Crane, 1983). Whether these early beekeepers were able to recognise honeybee diseases is not known but one can assume that they were aware of pests and predators attacking their colonies as is evident from the fact that Egyptians closed the entrances of their colonies. The small entrances gave the bees a better chance to ward off these pests and predators when they tried to enter the colonies.

As beekeeping developed, colonies were placed closer together and moved by the beekeepers from honey-flow to honey-flow. These manipulations inevitably increased the chances of colonies contracting and spreading pests and diseases because the colonies were placed under stress when handled or taken to 'unsuitable' areas. As colonies were placed closer together, drifting and robbing bees could enter neighboring colonies more easily, which in turn increased disease and pest dispersal between colonies and apiaries.

As Europeans started to colonise all corners of the earth they took the foodstuffs they knew from home with them. Once settled, they implemented their traditional farming methods, which included beekeeping. It is for this reason that European honeybees were taken to the New World and Australasia in the1620s (Crane, 1983). The Europeans that colonised Africa found that this continent had indigenous honeybees. These African races were good honey producers but they were also more defensive than the European races and this was probably the main reason why European queens were imported into southern Africa. The earliest record the author could find of the importation of European honeybees suggest that European races were imported in the 1800's (Attridge, 1917), but chances of even earlier importations are good. Since then numerous subspecies of honeybees have been imported to southern Africa (Fletcher, 1977; Hepburn & Radloff, 1998). We may never be able to determine whether these imported bees brought some previously unknown honeybee diseases to South Africa. However, this is likely as the introduction of the parasitic mite *Varroa destructor* into South Africa (Allsopp, 1997) is evidence of this kind.

HISTORY OF HONEYBEE DISEASES AND PARASITES IN SOUTH-AFRICA

The records of first discovery of diseases and parasites in South Africa are given. However, the author is of the opinion that many of them were present in this country long before they were recorded.

The early history of beekeeping in South Africa is not well documented. Apart from the rock paintings depicting some association with bees (Pager, 1973; Woodhouse, 1987) there are no records of pre-colonial beekeeping. These rock paintings depict the indigenous people of southern Africa robbing wild colonies but it is unknown whether they claimed and protected these wild colonies or only returned from time to time to rob (harvest) honey and/or brood. It seems probable that the wild nests were harvested regularly by knowledgeable tribe members, because many of the indigenous place-names reflect the importance of honey and honeybees (Tribe, 1997), but that no extra management ever took place.

Even before the first European settlers arrived in 1652 the early explorers gathered honey from the newly discovered continent as the inscription in the diary of Vasco da Gamma of his 1497 voyage describes: "On the next day after we had come to rest here … we went ashore … one of those men … was going about gathering honey on the moor …" (da Gama, 1497).

The first European settlers brought with them their ways of farming. These early settlers probably practiced beekeeping because they knew the value of honey as a sweetener and probably used it in brewing, baking and wine making. The first domesticated colonies were mostly kept in homemade hives or boxes (Attridge, 1908) but in time modern European standard hives were used (Anderson, 1985). These early European beekeepers probably used the knowledge they learned in their fatherlands and later taught others.

It was not until 1908 that the then Department of Agriculture published the first book on beekeeping in South Africa: "Bijenhoudery in Zuid-Afrika" (Attridge, 1908). From studying this publication it is apparent that bee diseases were of no serious concern at that stage, because the author has the following to say: (translated from High Dutch) "It is my privilege to announce in this book that South African bees have at this moment none of the serious diseases known to occur in other parts of the world. The climate (of South Africa) and resistance of the bees (to diseases) is without doubt the reason for this."
He goes on to explain that the “diseases”, that cause adult bee and/or brood mortality, that were shown to him by concerned beekeepers were the result of overheating, chilling or dearth. It must be taken into account that this publication was published soon after the discovery of the so-called "Isle of White disease", of 1905 in the United Kingdom (Bailey, 1964), which, for the first time, focussed the beekeepers’ attention on the importance of diseases in their colonies.

FIRST REPORTS ON PARASITES AND DISEASES IN SOUTH AFRICA

In the second edition of the book "Beekeeping in South Africa", Attridge (1923), (brother of author of the above-mentioned book) refutes all earlier statements, announcing that European Foulbrood (caused by the bacterium *Mellissococcus pluton*) was indeed present in the Western Province and Transvaal since 1908. He explained that he had to wait for positive identification before publishing this fact. This positive identification was done from

brood samples sent to the United States of America (Phillips, 1918). According to Lounsbury (1918 (a)) the then Chief, Division of Entomology, Department of Agriculture, received the news on the 31 March 1918. Lounsbury (1918 (b)) speculated that the EFB came into the country when honey was imported from England during the Boer-war. In a short report, Johannsmeier (1997) predicted that EFB was present in all apiaries in the country. However, he mentioned that there were only two severe outbreaks of this disease reported in South Africa, the first during the forties and a second in 1997. There is also a chance that South Africa has a different strain of foulbrood as suggested as early as 1911 by different bee pathologists ("Busy Bee", 1920).

Phillips (1918) also reported the presence of the virus causing Sacbrood in the same sample in which the EFB was identified.

After the positive identification of EFB and Sacbrood, the next bee disease to be identified was *Nosema apis*. It was first identified in 1920 (Fantham, 1920). Not long thereafter Skaife reportedly identified it from the Cape Province (Skaife,1954; Attridge, 1923). It seems as if the disease was not pronounced and was only mentioned occasionally when knowledgeable beekeepers saw mass honeybee deaths in their apiaries. Two such outbreaks were in 1954 in the Cape Peninsula (Skaife, 1954) and 1955 in Rhodesia* (Hayter, 1955). Many years went by without any mention until Buys in 1976 reported it again. In 1972 Buys described a nosema disease apparently killing brood and named it Brood nosema. His reports remain the only ones on a worldwide basis for Brood nosema up to this date.

The parasitic tracheal mite, *Acarapis woodi*, was also only recently reported by Buys (1995). He stated: "Over the years hundreds of bees from all over South Africa have been dissected and examined, amongst other things, for tracheal mites with negative results." However, this parasite was probably here longer, as a short article in the South African Bee Journal of 1922 (Anon.) indicates. In response to an article that appeared in the 'Farmer's Supplement' of the Sunday Times newspaper of 5 November 1922, a Mr. Le Mare is cited as writing: "The Isle of Wight disease, … , is also known to exist in South Africa. A couple of cases were found in Natal and the infected hives destroyed. The cause was traced to a small destructive mite which gets into the bees' breathing tubules." According to Le Mare he was able to identify the tracheal mite under a microscope. However, this discovery was not welcomed at all, especially in the light of the Isle of White

disease that was at that stage ravaging beekeeping in the United Kingdom, as can be seen from the response to this mentioned report. The writer responded by declaring: "To sum up, we believe we are correct in stating that no case of the Acarine disease caused by the mite *Tarsonemus woodi*, and known as Isle of Wight disease has ever been found outside the British Isles, and certainly not in South Africa." The writer ends his strong denial by making sure everyone understands that such claims will harm apiculture in the Union.

The first reference to calkbrood, caused by the fungus *Ascospaera apis*, is by May (1969), but it was not until 1999 (Swart & Rong) that it was positively identified. This condition was known to beekeepers but because it occurred erratically and did not cause any colony loss, it was largely ignored.

The parasitic mite *Varroa destructor* is the latest calamity to hit South African beekeepers. Allsopp first reported this parasitic mite in 1997 in the Western Cape Province. It has since been reported in all the provinces of South Africa.

The only other mites described in South Africa are *Miltiphus alvearuis* (Swart & Uekermann, 2001) and *Acarapis externus* (Eukermann, pers. comm.). Both these mites are not considered to be serious parasites. Lundie (1951) mentioned an unnamed external mite collected in the Cape Province.

In a recent survey, Davidson *et al*, (1999), reported Black Queen Cell Virus (BQCV), Acute Paralysis Virus (APV) and Sacbrood Virus (SBV) in South African bees. These viruses are rare and it is doubtful if any outbreak that caused mass mortality has ever occurred in this country. However, BQCV and APV are associated with colony mortality in countries where *Varroa* is present.

The search for other parasites and diseases has so far, thankfully, been negative. The most dreaded of these, American Foulbrood (AFB) is still unknown in South Africa as the latest report of Davidson *et al.* (1999) confirms. There have, however, been a few incidents where beekeepers and scientists reported the possible existence of the bacterium, *Paenibacillus larvae*, that causes AFB in South African colonies. (Allen, 1996; van der Merwe, 1956; Shimanuki, pers. comm.). However, Lear (1996) mentions a personal conversation with Prof. Ritter, a renowned honeybee pathologist of Germany,

who mentioned that AFB was present in a sample sent to his laboratory from South Africa. Ritter further explained that he knew that African bees were carriers of this bacterium without showing symptoms.

The protozoan, *Malpighamoeba mellifica*, is also still unreported in South Africa despite two major surveys conducted to determine its presence. The first was done during 1995 (Buys, 1993) and the second between 1997-1999 by the author and colleagues (Swart & Sandmann, 2001). However, a report by Buys (1993) confirmed amoeba disease in Zimbabwe, indicating that it is present in sub-Saharan Africa.

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NOSEMA DISEASE

INTRODUCTION

The microsporidium *Nosema apis* (Zander) was described by Zander in 1909 but it was White (1919) who proclaimed it to be a disease-causing endoparasite of honeybees. All microsporidia are spore-forming intercellular parasites that inject their sporoplasm into a host cell through a tubular structure - the polar filament (Weidner *et.al*., 1984). Microsporidian development is thus strictly intercellular (Vávera, 1976).

Although microsporidia have different life cycles, *N. apis* is tissue specific, and can only reproduce in the mid-gut epithelial cells of the honeybee (Liu, 1984). The *N. apis* spores are big, regular oval bodies 4 to 6 µm long and 2 to 4 µm wide. Once the spores are ingested and reach the ventricular lumen in the mid-gut they germinate in less than 12 hours (Bailey, 1955; Fries, *et.al*., 1992). The protozoan extrudes a hollow polar filament that penetrates an epithelial cell through which its germ is injected into the cell (De Graaf *et al.*, 1994). The protozoan then develops and multiplies in the cytoplasm of this host cell (Bailey, 1981).

Irrespective of the initial dose, the whole ventriculus becomes infected within 2 weeks after the initial spore ingestion, (Fries, 1988). As infected ventricular cells are shed into the gut lumen, newly formed spores are released and germinate, thus spreading the infections into other parts of the gut (Bailey, 1981; Steche & Held, 1981). The infection also spreads through autoinfection when spores germinated while still inside the cells where they were produced and then injected their sporoplasm into adjacent cells (Fries, 1989). The gut of heavily infected bees can contain up to 180 million spores (Cantwell, 1970).

Eventually spores will be voided with fecal matter. If this happens inside the colony re-infection may occur when the bees clean up the nest (Böttcher, 1956). Some spores will remain inside the hive or nest and may survive in a dormant state in fecal deposits for at least a year (Bailey, 1962).

Nosema causes disease of adult honeybees only, and infections have been reported throughout the world (Matheson, 1993). Workers, drones and queens can be infected (Anderson, 1993).

The seriousness of nosema disease differs within a colony, from colony to colony, apiary to apiary, and area to area (Doull & Cellier, 1961; Jaycox, 1960; Moeller, 1962, 1968; Mussen *et al*., 1975; Oertel, 1964). However, it seems as if the enzootic levels of infection stay more or less constant from year to year (Furgala *et al*., 1973). In New Zealand, Malone *et al*. (1992) showed that the mean number of spores per infected bee was high during initial infection but dropped and stabilised within eleven weeks to a infection of less than 4 million spores per infected bee.

Colonies placed under stress, such as constant hive manipulations, or bad weather conditions may be more prone to disease development (Goetze & Zeutzschel, 1959; Oertel, 1967).

SYMPTOMS

Some infected bees may not exhibit any symptoms or the symptoms exhibited on colony level could be similar to some viral infections, pesticide poisoning, or starvation. It is therefore impossible to determine infection beyond doubt by casual observation. A knowledgeable beekeeper may be able to distinguish between a healthy and heavily infected worker bee by looking at the colour of the midgut, but to accurately diagnose the severity of infection the content of the mid-gut must be studied under microscope (Bailey, 1981; Furgala & Mussen, 1990).

Most beekeepers only realize that their bees are diseased when colonies are noticeably affected. Colony symptoms are therefore often the first indication of nosema disease in an apiary. In colonies with high levels of infection bees may walk on the floor of the hive and on the ground next to the colony with distended abdomens and disjointed wings. According to Moeller (1978) crawling bees are only characteristic of the disease during the first few days of a heavy honeyflow. He speculated that the diseased bees might be too weak to handle heavy nectar loads. This weakness may also be as a result of malnutrition because Tomaszewska (1976) found that infected workers had lower levels of protein, lipids, glucose and fructose present in their bodies. Dysentery has for many years been given as a sure symptom of infection, but Moeller (1978) found colonies with high levels of infection without dysentery.

Because the effects of this disease are not obvious Kauffeld *et al*. (1972) suggested that colony dwindling, queenlessness, supersedure and reduced honey yields should be measured to estimate the true damage nosema infection had on a beekeeping operation. According to Kang *et al*. (1976) infected workers' life expectancy was reduced to between 22 to 44% of that of healthy workers. During spring and summer this might cause a shortage of experienced (older) foragers and thus the colony may not build up as quickly and vigorously as normal (Delaplane, 1998).

Infected bees do not fully develop their hypopharyngeal glands (Hassanein, 1952; Wang & Moeller, 1969, 1970, 1971) and feeding of larvae could thus be inadequate so that many may die or not develop properly. These resultant underdeveloped workers may not perform optimally, placing more strain on the colony. The worker force will therefore decline. In severe cases dwarf bees may be produced (Fyg, 1959).

AIM

The aims of this study were to: A) establish the distribution and severity of nosema disease within the African honeybee region of South Africa, B) compare the disease with the presence of the Cape Problem Bee and determine whether any correlation between the two problems exists.

MATERIALS AND METHODS

Beekeepers were asked to participate in the so-called “Capensis survey” and those who volunteered were asked to contribute 20 colonies that were randomly selected and thoroughly inspected for the duration of the survey. Prior to inspection, the beekeepers were interviewed and information on the history of the apiary, colonies, and migratory routes of the past year was gathered. The beekeepers were asked to point out colonies that they suspected of being diseased. In most instances the beekeepers could not single out diseased colonies and asked the surveyors to choose colonies by themselves. To increase the probability of finding parasitised or diseased colonies, the behaviour of

workers at the entrances of the selected colonies were observed. The surveyors selected any colonies with crawling or dead workers at or near the entrance for the disease sampling. Because such colonies were rare, disease samples had to be taken while the demographics for the 'Capensis surveys' were taken.

To determine nosema infection adult worker bees were collected from four of the 20 colonies. Colonies showing obvious disease symptoms were selected but if there were none (or not enough) then the samples were taken at colony no's. 5, 10, 15, and 20. The apiaries were sampled approximately every six months over a period of 18 months. The same colonies were again sampled every time, but if sampled colonies died out or were not found at the next sample session, then the colony with the number closest to the one previously sampled were used, i.e. if no. 10 was missing, then no. 9 or 11 was sampled.

Approximately 50 adult bees was selectively scooped into a 40-ml urine sample bottle, partly filled with 80% ethyl alcohol mixed with a few drops of glycerin. Older bees and those that appeared to be infected (as described previously) were targeted. Bees that appeared to be diseased where individually picked up by the fingers and placed in the sample bottle. If all bees seemed healthy, the sample was collected by positioning the opened bottle beneath bees that were adhering to a comb and the bees then scraped from the comb with the bottle. Bees were scrapped from the frame by turning it horizontally and collecting the bees from the underside of the comb by moving the bottle parallel with, just touching the comb. The bottle opening was held at a sharp angle of approximately 25° to the comb surface. Each sample were closed, individually marked and taken to a laboratory for further examination.

Twenty bees per sample were randomly examined following the procedure of Doull and Cellier (1961) who found that sample sizes of 20 to 25 bees eliminated sampling error in and between colonies during surveys for *N. apis*. Since the aim of the survey was to establish the presence or absence of the disease the more complex methods described by Cole (1970) or van Lear & de Wael (1981) were not utilized. Instead the testing methods used by the USDA Honeybee Disease Testing Laboratory and described by Shimanuki and Knox (2001) were used.

The abdomens of twenty randomly selected bees were removed by pinching them off with a forceps (no. 10). These were then placed in a clean mortar and covered with

approximately 15-ml water and mashed with a pestle. Three drops of this mixture were collected with a micro-pipette at the bottom center of the mixture. The drops were placed on a microscope slide and covered with a cover slip. The slide was then examined at 400 X magnification for spores. When spores were detected they were graded as a low, medium, or high, infection, where 'low' was less than 10 spores seen, 'medium' between 10 and 50, and 'high' more than 50.

RESULTS

A total of 56 apiaries were surveyed during the 18-month period. A total of 569 colonies were sampled during the entire time with 209 colonies sampled in the first survey, 208 in the second and 153 in the third (see Appendix A). The decrease in the number of samples as time went by was mainly due to the die-off of colonies as a result of the Cape Problem Bee (see Appendix C).

Nosema spores were found in 166 colonies (29%) of the 570 colonies tested. In the first survey 48 (23%), in the second 66 (32%) and in the third survey 52 (34%) of the colonies were infected respectively. On average 7 out of the 24 colonies were infected.

The percentages of nosema infected colonies per region for the entire survey period are given in Table 1. The subdivitions within provinces are explained in the “Materails and Methods” section of the “Cape Problem Bee” chapter.

Infection within sedentary- and migritory operations was calculated and is shown in Table 2. In the first survey more sedentary operations were infected than migratory ones (22 to 19), but this changed in survey two (30 to 36) and significantly in survey three (18 to 34).

Table1: Colonies infected per region – *Nosema apis*

Survey No.	Region	NC	FS	SG	NG	NP	MP	NKN	SKN	Total	av.	F-Test
1	Total surveyed	12	28	44	28	22	32	20	23	209	**26.13**	
	Total infested	**1**	**0**	**9**	**7**	**2**	**9**	**3**	**17**	**48**	**6.00**	1:2
	% infested	**8.3**	**0.0**	**20.5**	**25.0**	**9.1**	**28.1**	**15.0**	**73.9**	**23.0**	**22.49**	0.8005
2	Total surveyed	12	28	39	24	24	29	28	24	208	**26.00**	
	Total infested	**1**	**1**	**8**	**6**	**18**	**12**	**15**	**5**	**66**	**8.25**	2:3
	% infested	**8.3**	**3.6**	**20.5**	**25.0**	**75.0**	**41.4**	**53.6**	**20.8**	**31.7**	**31.03**	0.8005
3	Total surveyed	11	20	31	16	16	6	27	26	153	**19.13**	
	Total infested	**0**	**0**	**10**	**7**	**6**	**3**	**9**	**17**	**52**	**6.50**	1:3
	% infested	**0.0**	**0.0**	**32.3**	**43.8**	**37.5**	**50.0**	**33.3**	**65.4**	**34.0**	**32.78**	0.0127
	Total (surv.)	35	96	122	84	60	79	75	73	570		
	Total (inf.)	**2**	**1**	**27**	**20**	**26**	**24**	**27**	**39**	**166**		
	% infested	**5.7**	**1.0**	**22.1**	**23.8**	**43.3**	**30.4**	**36.0**	**53.4**	**29.1**		

NC = Northern Cape, FS = Free State, SG = southern Gauteng, NG = northern Gauteng, NP = Northern Province, MP = Mpumalanga, NKN = northern Kwa-Zulu Natal, SKN = southern Kwa-Zulu Natal

Table 2: Colonies infested of sedentary vs. migratory beekeepers

Survey	Sedentary	Migratory
1	29	19
2	30	36
3	18	34
SD	6.66	9.29
F test		**0.68**
Total	**77**	**89**

DISCUSSION

The effects of infection on colony performance could not be determined from the data collected because only the presence or absence of the Nosema spores was determined and production records could not be supplied by the participating beekeepers.

Buys (1976) reported that all hives he examined in an isolated apiary on Robben Island were infected "to some degree at some stage during the year." His data showed that an average of 20% of all colonies was infected during the year. In this survey we found that

an average of 29% colonies infected. Whether the higher infection was a result of the capensis problem could not be ascertained.

Nosema was present in all regions but at significantly higher levels in regions where commercial beekeepers operated. In the drier areas of the Free State and Northern Cape, the disease was lowest (less than 1% of the total). Whether it was due to the lower humidity or hot summer temperatures could not be determined. It is generally accepted that nosema is more prevalent in colder areas and that infection severity increases when workers are confined to the hive for prolonged times (Moeller, 1978). Therefore, in the summer rainfall region of South Africa where the winters in most parts are temperate an increase of infection at the end of winter and during early spring was not expected, nor found. Instead, the percentage of colonies infected increased during the survey period from 22% to 31% to 34%. This coincided with the increase of take-over by Capesis Problem Bees. The collapse of colony cohesion after take-over results in a breakdown of pheromonal control that causes a breakdown of social order. This results in poor sanitation, ventilation and feeding of larvae. These conditions should favor nosema spread within a colony. Heavily infected colonies within an apiary will then act as contaminants for healthy colonies.

It is interesting to note that the highest incidences of the disease were in the forestry areas of South Africa where *Eucalyptus grandis* are predominantly planted i.e. southern KwaZulu Natal [53%], Nothern Province [43%], northern KwaZulu Natal [36%]. The higher humidity, lack of direct sunlight or higher relative humidity within the hives when they are placed inside the plantations may be conducive to the disease. The lack of direct sunlight on the colonies placed in these plantations may hinder the proper regulation of heat and humidity inside their nests and stifle the colonies, placing them under unnecessary stress. According to Pack (1977) stress is the major reason for increased nosema infection in the temperate and subtropical regions of Australia.

During the first survey more sedentary than migratory beekeeping operations were infected. However, this changed during the second and third survey with more migratory beekeeping operations infested. This may indicate that the continual stress placed on migrated colonies do increase the risk of colonies contracting nosema as Pack (1977) indicated.

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TRACHEAL MITES

INTRODUCTION

This parasite was first described by Rennie *et al*. (1921) from honeybees in Great Britain and named *Tarsonemus woodi*. In the same year, Hirst (1921) revised the classification of some mite genuses and placed this mite in the genus Acarapis, thus changing the name to *Acarapis woodi*.

The discovery of this parasitic mite in honeybees became the topic of discussion for many years when many colonies died on the Isle of Wight. A historical review of this so-called 'Isle of Wight' disease by Bro. Adam (1968) is summarized: "The first colonies that died was reported in 1906, but the cause was ascribed to bee paralysis. At first the disease were not taken seriously, but as the news of the many dying colonies increased, the possibility of a new disease was realized. By 1909 all colonies that were on the island since 1904 were dead. In that same year the symptoms were seen in colonies on the mainland (Great Britain). Within the next 10 years almost all colonies on the mainland also died." Bro. Adam further explained that at first - during 1913 - scientists working for the Board of Agriculture announced that the colonies succumbed to *Nosema apis* but that Anderson (1916) disagreed.

According to Bro. Adam, Rennie started studying the disease in 1917 and in 1923 published his results, showing that 99% of stocks reported to be diseased harbored the tracheal mite *Tarsonemus (Acarapis) woodi*. However, Bailey (1964) also studied the 'old' bee literature of the time prior to, during, and after the epidemic and came to the conclusion that although tracheal mites was associated with the disease, it could not conclusively be linked to the deaths of the colonies. He suggested that abiotic factors, mainly severe cold and subsequent dearth; beekeepers using dangerous and poisonous concoctions to cure their colonies; and possible bacterial and viral infections, were all probable factors that led to the mass mortality of colonies. To support his theory he mentioned that similar mass colony mortality with similar symptoms had been reported in other countries even before the Isle of Wight incidence.

Whether tracheal mites were the main cause of the Isle of Wight colony deaths, or not is now only of historic importance, however, this calamity placed a focus on the importance of parasites and honeybee diseases. It also led to the first legislatory prohibitions on the importation of honeybees into countries. But despite these regulations this parasite in time spread to all continents accept Australia.

Three *Acarapis* mite species have been described to occur on honeybees. These mites can positively be identified solely on habitat (Dafinado-Baker & Baker, 1982). Tracheal mites occur exclusively in the prothoracic tracheae of the honeybee and will only leave the tracheas to disperse between bees. The tracheal mites afflict only adult honeybees of all castes. The *A. woodi* female is 143 - 174 µm in length and the male 125 - 136 µm. The body is oval, widest between the second and third pair of legs, and is whitish or pearly white with a shining smooth cuticle. It has an elongate, beak-like gnathosoma with long blade-like styles (mouthparts) for feeding. Delfinado-Baker & Baker (1982) did the most recent and thorough morphological description of this mite and its close relatives.

Mating probably occurs within the trachea and mated females disperse between bees. It seems highly unlikely that a female mite that has already started to oviposit will migrate from one bee to another (Gary *et al*., 1989). When a mite transfers from one bee to another, it will only move directly to the trachea if it landed close to it, but mostly it will first move to the wing auxiliaries, then into the vestibule of the prothoracic spiracles before entering the trachea. (Hirschfelder & Sachs, 1952; Royce *et al*. 1988).

Although older bees can be infested (Gary *et al*., 1989; Smith *et al*., 1991), mites will almost always infest bees that are less than 10 days old (Giordani, 1977; Gary & Page, 1987; Lee, 1963; Morgenthaler, 1930; Phelan, *et al*., 1991). The first female mite to enter the trachea has no preference to where she will settle (Giordani, 1977). As soon as she feels comfortable she punctures the tracheal walls and starts feeding on the hemolymph of the bee. Soon thereafter (< 1 day) she will start laying eggs (Gary *et al.*, 1989). She lays 5 to 7 eggs over a period of 3 to 4 days (Morgenthaler, 1931). After 3 to 4 days the eggs hatch and the mites follow a life cycle of a six-legged larva, then an eight-legged nymph that moults into an adult after about 11 to 12 days for a male and 14 to 15 days for a female. (Henderson & Morse, 1990).

The population of *A. woodi* varies seasonally (Otis *et al.*, 1986), within colonies, apiaries and areas.

SYMPTOMS

There are no characteristic symptoms for this disease. Symptoms often associated with infestation are the crawling of workers, fluttering and distended abdomens (Bailey, 1969, 1984). An affected bee could have disjointed wings (Shimanuki & Knox, 2000). Because the mites pierce the tracheal walls for feeding the flight muscles can be injured through the direct mechanical action, which in turn may damage some nerves that may be important for flying (Anderson, 1928, Dalfinado-Baker, 1988; Komeili & Ambrose, 1991). The uptake of hemolymph may also indirectly cause injury to the flight muscles by depriving them from blood (Morison, 1927). The mites may also transmit fatal viruses (Bailey *et al*., 1983; Shimanuki, *et al*., 1995) or the bee may die as a result of stress when the bee's immune system overreacts to the parasitism (Hung, *et al*., 1995). However, absence of these symptoms does not necessarily imply freedom from mites (Shimanuki & Knox, 2000).

There are conflicting reports on the economic impact of this pest. Some authors reported a reduction in honey production (Eischen *et al*. 1989; Guzman-Novoa & Zozaya-Rubio, 1984), and others deficient pollination (Otis, 1990), but heavily infested colonies can produce average and even above-average honey crops (Giordani, 1977). Gary and Page (1989) could find no difference in the foraging behaviour, or efficiency of infested compared to non-infested workers. They could also find no difference between the longevity of these bees. Harrison *et al*. (2001) could not show that infested bees had any difficulty in breathing or a reduced flight ability, and Bailey (1958) reported that infested young bees showed no abnormal behavior, but claimed reduced longevity (Bailey, 1965). It would seem that colonies that are over-wintered are the most susceptible because high mortality and a poor brood production has often been reported (Bailey, 1958, 1961; Bailey & Lee, 1959; Cox *et al*., 1988; Eischen, 1987; Furgala *et al*., 1989; Gruszka, 1987; Komeili & Ambrose, 1990; Maki *et al*., 1988; Otis & Scott-Dupree, 1992; Royce & Rossignol, 1990).

It is difficult for the beekeeper to recognise colonies that are infested because the effects of this parasite are very subtle. It has been reported that colonies with moderate to heavy

infections rear less brood during peak brood rearing times, resulting in under-optimum colonies (Eischen, 1987). These infested colonies apparently consume more stored honey then healthy colonies, reducing the harvest, but also consuming possible winter stores (Bailey & Ball, 1991; Otis & Scott-Dupree, 1992). In some instances heavily infested colonies may abscond, leaving large numbers of dead and dying (crawling) workers near the hive entrance (Bailey, 1969, Royce *et al*., 1991; Thoenes & Buchmann, 1992). However, other conditions, such as pesticide poisoning and Bee Paralysis, may also cause these symptoms. A less obvious symptom is the dwindling of the adult population, and ultimately the colony that, according to Maki *et al*., (1988) is correlated with the shortened life-span of adult honeybees.

AIM

The aims of these experiments were to: A) establish the distribution and severity of tracheal mites within the African honeybee region of South Africa, B) compare and determine any correlation between the disease with the presence of the Cape Problem Bee.

MATERIALS AND METHODS

The same colonies as those selected for the Nosema samples were also sampled for possible tracheal mites infestations (see previous chapter).

Each brood frame was individually lifted out of the brood chamber and the adhering bees shaken back into the chamber. The frames were then placed in another brood-chamber placed in front of the hive being inspected. This was done to divert the returning foragers away from the colony being inspected. After all the brood-frames were removed a sample of approximately 50 adult worker bees were randomly collected in a 40-ml urine sample bottle half filled with 75% ethyl alcohol by scooping them from the inside side of the brood-chamber. The sample was taken inside the brood chamber to increase the chances of collecting younger bees because most foragers had by that time clustered onto the frames in the second brood-chamber.

To examine the tracheae the methodology described by Shimanuki & Knox (2000) was followed. Twenty worker bees per sample were randomly taken from the sample bottle and each bee dissected by holding her by the thorax between thumb and forefinger and scraping off the head and first pair of legs with a scalpel (no. 4 blade). A thin slice (± 0,5 mm) of the front of the thorax (the color) was then cut from the ventral to the dorsal side of the thorax after pinning the bee onto a piece of cardboard. The disks of five bees were then transferred onto a microscope slide and a few drops of 85% lactic acid were placed onto the muscle tissue. This was done to dissolve the muscles and to make the material more transparent so that the tracheas could be distinguished and studied with more ease.

With the aid of a dissecting microscope at 10 X magnification, muscle tissue was carefully separated and the tracheas removed using a forceps (no. 10). The tracheas were then transferred and carefully placed onto a second microscope slide. A drop of lactic acid was added to enhanced contrast. The preparation was then covered with a cover slip. The tracheas were individually examined at 40 X magnifications.

RESULTS

A total of 56 apiaries were surveyed during the 18-month period. Samples from 569 colonies were collected with 209 analised during the first survey, 208 during the second, and 153 during the third (see Appendix B). The decreases in the amount of samples taken were mainly due to the die-off of colonies as a result of the Cape Problem Bee (see Appendix C).

Infestation of trachea mites was found in a total of 64 colonies (10,3%) over the 18-month period. In the first survey 28 (13,4%), in the second 23 (11,1%), and in the third 12 (7,8%) colonies were infested respectively.

The percentage of colonies infested with tracheal mites throughout the study period in the different regions are given in Table 3. The subdivitions within provinces are explained in the “Materails and Methods” section of the “Cape Bee Problem” chapter.

There were no significant defferences between the infestation levels between surveys. However, there was a significant difference between infestation between sedentary and

migratory beekeepers. It was clear that colonies in sedentary beekeeping operations was more suseptable to infestation.

Table 3: Colonies infested per region – *Acarapis woodi*

Survey No.	Region	NC	FS	SG	NG	NP	MP	NKN	SKN	Total	av.	F-Test
1	Total surveyed	12	28	44	28	22	32	20	23	209	**26.13**	
	Total infested	**4**	**5**	**8**	**1**	**2**	**0**	**7**	**1**	**28**	**3.50**	1:2
	% infested	**33.3**	**17.9**	**18.2**	**3.6**	**9.1**	**0.0**	**35.0**	**4.3**	**13.4**	**15.17**	0.5530
2	Total surveyed	12	28	39	24	24	28	28	24	207	**25.88**	
	Total infested	**5**	**1**	**3**	**2**	**0**	**1**	**4**	**7**	**23**	**2.88**	2:3
	% infested	**41.7**	**3.6**	**7.7**	**8.3**	**0.0**	**3.6**	**14.3**	**29.2**	**11.1**	**13.54**	0.4003
3	Total surveyed	11	20	31	16	16	6	27	26	153	**19.13**	
	Total infested	**1**	**3**	**1**	**0**	**1**	**0**	**1**	**5**	**12**	**1.50**	1:3
	% infested	**9.1**	**15.0**	**3.2**	**0.0**	**6.3**	**0.0**	**3.7**	**19.2**	**7.8**	**7.06**	0.5155
	Total (surv.)	35	96	122	84	60	79	75	73	569		
	Total (inf.)	**10**	**9**	**12**	**3**	**3**	**2**	**12**	**13**	**64**		
	% infested	**28.6**	**9.4**	**9.8**	**3.6**	**5.0**	**2.5**	**16.0**	**17.8**	**11.2**		

NC = Northern Cape, FS = Free State, SG = southern Gauteng, NG = northern Gauteng, NP = Northern Province, MP = Mpumalanga, NKN = northern Kwa-Zulu Natal, SKN = southern Kwa-Zulu Natal

Table 4: Colonies infested of sedentary vs. migratory beekeepers

Survey	Sedentary	Migratory
1	18	10
2	14	9
3	10	2
SD	4	4.36
F test		**0.91**
Total	**42**	**21**

DISCUSSION

The mite seems to have spread quite rapidly in South Africa since its discovery by Buys (1995). He found only two bees infested with mites in the summer rainfall region (at Standerton and Greytown) out of 3030 he sampled. These surveys were done only 5 years later and mites were found in apiaries throughout the summer rainfall region.

This parasitic mite was present in all regions, although in low numbers in three most northern regions. The infestation within a region showed that the northern Cape (29%) was significantly more infested than any other region. Whether this is as a result of a different honeybee haplotype in that region that are more susceptible, or if it is climatic factors (hot and dry summers and mild winters), was not established.

The greater Kwa-Zulu Natal (SKN with 18% and NKN with 16%) region followed with the other regions significantly less infested. This region proofed to top the list as the region with the most disease problems. The author can only speculate about the reasons for this. The short protracted nectar-flow in the Eucalyptus forests may place more stress on these colonies than in other areas. Outside of this nectar-flow period the colonies have to survive on sustentation nectar sources. The colonies shrink and more adult bees get infested because less brood is produced. During the *Eucalyptus* flow, for a short period, there are ample young bees that are re-infested. In other regions the colonies can utilise more nectar flows and therefore the ratio of old to young bees remain more balanced. Another explanation could be the same as that given for nosema infection, and that is that higher stress levels are created when the colonies are placed inside the Eucalyptus forests.

On average 2.6 colonies were infested, which means that 7% of all apiaries were infested. The number of colonies infected diminished over the survey period. This is not in agreement with results from Bailey (1958) and Dawicke *et al*. (1992) who found higher infestation during the winter months. They explained that if one assumes that the percentage of infection stayed more or less constant there is less bees within a colony during winter than summer, increasing the chances of collecting bees infected. Alternatively, in summer and spring when a lot of workers is out foraging, a sample collected inside the colony may represent only young bees that has not been infested or has low a infestation.

The level of infection differed from bee to bee, indicating a variance in levels of infection within a colony.

Sedentery beekeeping operations was significantly more infested. The best guess why this is so, is that the periods of egglessness when colonies are migrated and the queen stops laying eggs, or when the colony is stressed and they stop feeding the queen who

then stops laying eggs, may create sufficient breaks in brood rearing to hamper a constant supply of young workers for re-infestation. The loss of foragers when bees are moved may also play a part.

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THE CAPE BEE PROBLEM

INTRODUCTION

The so-called ‘Cape Bee Problem’ (also referred to as the ‘Capensis Problem’) causes the death of thousands of African honeybee colonies in South Africa. It is not a disease, *per se*, but is equally destructive to colonies that succumb due to a breakdown of the social structure when workers of the Cape honeybee, *Apis mellifera capensis* (Eschscholtz), enter colonies of the African honeybee, *Apis mellifera scutellata* (Lepeletier), and start laying eggs in the presence of the invaded colonies' queen.

The Cape honeybee is localized at the southern tip of Africa (Ruttner, 1975, 1976 (a)) and its distribution coincides more or less with that of the Fynbos biome (Hepburn & Crewe, 1991; Hepburn & Jacot Guillarmod, 1991; Tribe, 1983). The African honeybee is found north of this sub-species and occupies a large area that stretches up the western coast to central Namibia, following the southern coast to southern Mozambique and covering the Great Rift Valley with its northern upper most border in central Ethiopia (Ruttner, 1975; Hepburn & Radloff, 1998). A changing but stable hybrid zone naturally divides these two races (Hepburn & Crewe, 1990, 1991).

When a honeybee colony becomes queenless a few workers usually develop active ovaries and turn into laying workers. They are able to lay eggs but do so haphazardly, laying more than one egg per cell in a non-orderly fashion, even placing some on the side of cell walls. Most of these eggs will not hatch because workers will eat many and others will dehydrate. Eggs that do hatch into young larvae will be neglected and many will desiccate.

The eggs of laying workers are furthermore not fertilized because the workers cannot mate and therefore carry no sperm. Subsequently the eggs contain only one set of chromosomes - they are haploid - and their offspring are drones. However, the Cape honeybee is different in this regard because their laying workers have the unique ability to produced diploid (worker) offspring without mating. In 1909 Onions (Onions, 1912) described this unique feature of the Cape honeybee but it took almost seven decades

before Ruttner (1977) described the process of thelytokous parthenogenesis that biologically explained how this was possible.

FIRST REPORTS OF THE CAPE BEE PROBLEM IN SOUTH AFRICA

This unique ability of the Cape honeybee was regarded as just a peculiarity (Ruttner, 1976 (a,b), 1977) and not seen as a threat to other honeybee races. This all changed, however, when Cape honeybee colonies were moved on two occasions between 1977 and 1979 to research apiaries in Pretoria that was situated well into the African honeybee area (Johannsmeier, 1983). The devastating results of keeping the two races in the same apiary became apparent when colonies that were queen-right just a short time before became inexplicably queeless and laying workers developed. Attempts of natural requeening within the colonies were unsuccessful and soon the colonies started to dwindle. Sensibly the researchers acted quickly, killing all the *A. m. capensis* colonies and *A. m. scutellata* colonies that harbored laying workers. Unfortunately the problem was not eradicated as more colonies expressed the same symptoms. The researchers then killed all the colonies in the apiary and those in the vicinity and eradicated the problem that way. Although this was not the only introduction of Cape honeybees into the African honeybee area (Lundie, 1954), it was the first time that *A. m. capensis* laying workers was reported to spread into, and cause the demise of so many *A. m. scutellata* colonies.

THE CURRENT PROBLEM

After the above-mentioned 1977 incident beekeepers were constantly warned about the possible catastrophic results that the introduction of Cape honeybees into the African honeybee area could have. However, in 1992 a beekeeper from Gauteng reported black laying workers in some of his colonies. These laying workers were identified as *A. m. capensis* bees (Allsopp, 1992; Johannsmeier & Magnuson, 1992).

The Government, researchers, and beekeepers reacted quickly and decisively and tried to eradicate the problem by killing all infested and possible infested colonies. This drastic measure was promulgated as a Government Notice (R 159, 1993) under Act 36 of 1983.

However, the problem was already widespread and this effort to eradicate it was unsuccessful (Allsopp & Crewe, 1993).

The government regulation ordering the initial killing of infested colonies was amended (Government Regulation R1674 of 1998) and beekeepers were instructed to continue killing Cape Problem Bee colonies. One has to assume that this regulation is keeping the problem in check, but to date the Cape Problem Bees has not been eradicated. Beekeepers report that infestation seemed to reappear almost cyclically even though infested colonies were regularly culled. Some beekeepers reported that they have eradicated the problem just to retract their statement within months, declaring that the problem was as damaging as ever. As one commercial beekeeper (De Klerk, pers. com. 2002) explained: “We have to catch 1200 trap swarms a year to be able to farm with 1000 colonies”. His statement is not uncommon and the author estimates that commercial beekeepers have to annually replace between 80 and 150% of their colonies (natural die off and culling) as a result of the Cape Problem Bee.

THE TAKE-OVER

When a colony that has been taken over by Cape Problem Bees is placed in an apiary and surrounded by pure *A. m. scutellata* colonies, the invasion happens gradually. A single, or few, Cape Problem Bee workers will enter the pure *A. m. scutellata* colonies and start to lay eggs away from the queens' egg laying area. It is only after this brood has hatched that the problem becomes apparent (Swart, 2001).

Worker bees can spread incidentally between colonies in various ways, and any action that will cause mingling between bees from different colonies, such as when they are routinely worked or when colonies are moved or migrated heightens the risk of intra-colony exchange of bees. The workers stray as a result of disorganization or interference of their flight path by the beekeeper. The beekeeper may assist in the spread of the problem by exchanging brood frames containing laying worker brood or amalgamating colonies. However, Johannsmeier (1983) reported that Tribe observed ‘*A. m. capensis*’ workers entering *A. m. scutellata* colonies when there was no colony disturbance and he suggested that the invasion spread through actively flying bees and that casual drifting was unimportant. The author has also observed this behaviour. Neumann (2001) in an

experiment in the natural distribution area of *A. m. capensis* has shown that *A. m. capensis* workers 'drifted' more into *A. m. scutellata* colonies than *vice versa*. It therefore seems as if *A. m. capensis* workers may rather target, than accidentally drift, into *A. m. scutellata* colonies. This may appear to be an almost parasitic behaviour. However, Cape Problem Bees seem to be unable to penetrate into the wild (over long distances) on their own accord (Swart & Sandmann, 2002). It therefore seems as if Cape Problem Bees will only deliberately enter colonies in close proximity, or looking at it in another way, can only survive if an uninfected *A. m. scutellata* colony was close-by to invade.

Queenlessness or situations where the pheromonal control of the queen is restricted will promote take-over. Heavy feeding, that promotes queen rearing in *A. m. scutellata*, also leads to the development of laying workers in *A. m. capensis*, this despite the presence of eggs and young brood (Hepburn, 1992(b)). Adding more than two supers will create a hive with a area too large for the queen to control sufficiently with her pheromone, and also leaves ample area for a Cape Problem Bee worker to lay eggs undetected (Swart & Sandmann, 2002).

When *A. m. capensis* workers are introduced into a colony of another sub-species such as *A. m. scutellata*, their ovaries become active and eggs are produced (Hepburn, 1992(a)). While this happens they also produce queen substance, 9-oxo-2-trans-decenoic acid (Ruttner *et al.*, 1976). The brood in queenless *A. m. capensis* colonies are heavier than brood reared in queen-right colonies (Anderson *et al.*, 1971). This behaviour is amplified when *A. m. capensis* brood is placed in *A. m. scutellata* colonies. The *A. m. capensis* larvae are fed more royal jelly than their *A. m. scutellata* nest-mates (Beekman *et al*., 2000). When they hatch they are thus bigger and more queen-like than the other workers. The combined presence of these ‘pseudo-queens’ probably dominates the pheromone bouquet of the colony leading to the killing or exile of the original (*A. m. scutellata*) queen.

As with any laying worker these pseudo-queens are not good at laying single eggs, centrally in an upright position, at the bottom of the cell. The multiple scattered eggs are usually the first indication to beekeepers that something is amiss in their colonies.

THE COLLAPSE OF TAKEN-OVER COLONIES

Despite the many eggs being laid by the Cape Problem Bees only a few will reach maturity and of these very few - if any - will act as normal worker bees, resulting in a collapse of general colony cohesion. The *A. m. scutellata* forager force naturally diminishes as they die off. No new foragers are produced, and the food stores are depleted within the nest. The aging *A. m. scutellata* workers cannot feed the remaining larvae and brood rearing ceases. At this stage many laying worker eggs are usually seen, most of them shriveled, and only a few – usually dead – capped brood cells. Secondary diseases such as European Foulbrood, and the breakdown of general sanitation are usually visible. After about 50 days all that remains is a few starving black laying workers/pseudo-queens (unpublished data).

It has been reported that these remnant swarms invade other colonies (Johannsmeier, pers. comm., 2002). However, the author is of the opinion that this method of invasion is uncommon. If colonies were often invaded *en mass* the take-over would not have been so subtle.

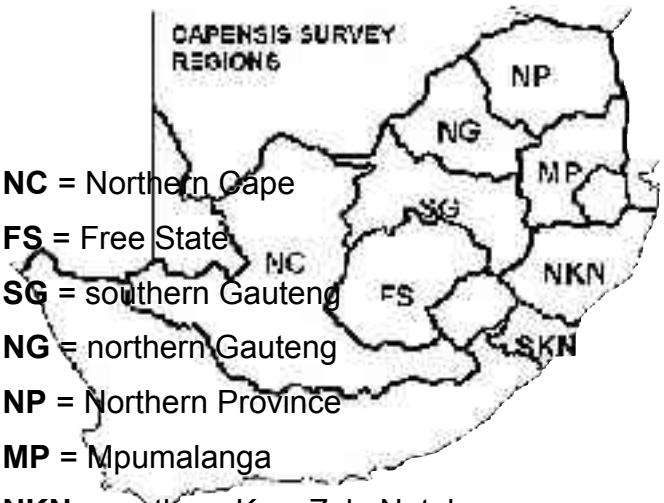
AIM

The aim of this study was to determine the severity of the Cape Problem Bee in the summer rainfall region. This was done by surveying the colonies of participatory beekeepers on three occasions. The surveys were done to determine a) the area and spreading of the problem, b) to find out whether there was a difference in take-over between sedentary or migratory beekeeping practices.

MATERIALS AND METHODS

The summer-rainfall region of South Africa was divided into eight regions: Northern Province, Mpumalanga, northern Gauteng, southern Gauteng, northern Kwazulu-Natal, southern Kwazulu-Natal, Free State and Northern Cape (Fig. 1). These regions conformed more or less to the provinces, but the areas were selected taking into account the major

beekeeping areas and routes migratory beekeepers most often used. The migratory beekeepers in each region operated mostly within an area and seldom moved colonies to another region.



NC = Northern Cape
FS = Free State
SG = southern Gauteng
NG = northern Gauteng
NP = Northern Province
MP = Mpumalanga
NKN = northern Kwa-Zulu Natal
SKN = southern Kwa-Zulu Natal

Figure 1: Map showing survey areas in the summer rainfall region

Migratory as well as sedentary beekeeping operations were surveyed. If colonies were moved away from the original apiary, no matter how short a distance, they were classified as ‘migratory’, because the bees were subjected to conditions associated with migratory beekeeping. Participatory beekeepers had to have at least twenty colonies in the apiary used for the survey. The colonies were hived in standard Langstroth hives. Some beekeepers used queen excluders while others didn't. During the first survey each colony was individually numbered on the front using a permanent ink marker pen.

Prior to inspection the beekeeper was interviewed and information on the history of the apiary, colonies, and migratory routes of the past year was gathered. The beekeepers were asked to point out colonies that they suspected of being taken-over or diseased. If they could not point out specific colonies the behaviour of workers at the entrances of the colonies was observed and colonies where dead or fighting bees were seen in front of the hives were selected. At each apiary twenty colonies or those remaining from the previous survey were thoroughly inspected on three occasions ± six months apart.

DISSECTION DATA

The sampled bees were placed in a freezer until they could be dissected. Twenty bees were taken from the sample bottle, their abdomen and scutellum colour visually graded from 1-4; 1 being yellow and 4 being black. The abdomen of the bee was thereafter horizontally cut with an “cornea-sissor” to expose the intestines. The ovaries were carefully removed using a forceps (No. 10) and mounted on a microscope slide. A drop of glycerin was placed on the ovarioles to enhance contrast. The left and right ovarioles were counted under a stereo microscope at 15 X magnification and a grade of development designated. The grade of development was ranged from one to four as follows: 1 = ovary with no development, 2 = ovary contain some milky substance, 3 = egg inclusions seen, 4 = eggs visible.

The mean- and maximum number of ovarioles of the dissected 20 bees per colony was determined and taking into account the status of ovariole development, the colony was classified as ‘scutellata’, ‘hybrid’, or ‘capensis’ (see Table 5) as modified from Hepburn & Crewe (1990).

Table 5: Racial Classification of Colonies using dissection data

Classification	Ovariole development	Stage IV	Mean number of ovarioles	Max. number of ovarioles
Scutellata	No	No	≤ 5	≤ 8
Scutellata Laying Worker	Yes	Yes	≤ 5	≤ 8
Hybrid	No	No	> 5	≤ 10
Hybrid laying worker	Yes	Yes	> 5	≤ 10
Capensis + Cape Problem Bee	Yes or No	Yes or No	> 10	> 11

INSPECTION DATA

Demographics of each colony were taken during the survey. Before a colony was opened the activity at the entrance of the colony was noted. Dead or fighting bees are an indication of early take-over (Allsopp, 1992). The colony was then opened. If there were supers on the hive the bees were shaken into the brood chamber. The brood-frames were then individually lifted from the brood chamber and inspected. The bees adhering to the

frames were scrutinised and then shaken into the brood chamber, and then the frame was placed in a second brood chamber and covered with a cloth to prevent robbing. Once all the frames were removed the bees were carefully and thoroughly inspected to find the queen. If a queen was present she was marked with Tipp-ex® with a different colour for each survey so that her presence throughout the survey could be determined. Once this was done the colony was reassembled. The inspections could not discriminate between hybrids and pure races and therefore the colony was only classified as either 'capensis' or 'scutellata' (see Table 6).

Table 6: Racial Classification of Colonies using Inspection Data

	Capensis	**Scutellata**
Colour of bees	Abdomen and scutellum = black	Abdomen and scutellum = yellow
Brood pattern	Scattered	Normal
Multiple eggs in cells?	Yes	No
Where?	Side of walls or bottom of cells	Bottom of cells
Cappings raised/flat?	Raised	Flat
Describe queen cells	Chewed open or cryptic	Normal
Queen present?	No or not sure	Yes or not sure

RESULTS

GENERAL SURVEY RESULTS

A total of 56 apiaries were surveyed during the 18-month period. In the first survey 1005 colonies in 61 apiaries were surveyed. In the second survey 803 colonies in 57 apiaries were sampled. Nine new apiaries were added but 13 apiaries were not surveyed again due to entire apiary loss or beekeepers unable to continue with the project. In the third survey 458 colonies in 41 apiaries were surveyed. This time 24 apiaries were not surveyed again mainly due to total colony loss. The devastating effect of the Capensis problem could already be seen because 35 apiaries were already completely wiped out during the interval of approximately six months between surveys.

Colony-losses of all apiaries, excluding those where beekeepers stopped participating, are shown in the tables 1 and 2. The figures shown in round brackets are the percentage differences per survey, and the figures in the square brackets are the total percentage

differences. Even with the addition of apiaries to 'fill-up' lost colonies during the second survey, 63% of all colonies were lost by the third survey. A fourth survey was planned but so few colonies were left after the third survey that it was deemed a waste of money to continue.

Table 7: Dissection data of colonies surveyed

	1st survey	2nd survey	3rd survey
Capensis	532	440 (-17.3%)	259 (-41.1%) [≅-51%]
Scutellata	473	357 (-24.5%)	197 (-44.8%) [≅-55%]
Hybrid	0	6	2
Total	1005	803	458
Colony loss (% lost)	(0%)	202 (-20%)	345 (-43.1%) [≅-63%]

Table 8: Inspection data of colonies surveyed

	1st survey	2nd survey	3rd survey
Capensis	48	35 (-27.1%)	24 (-31.4%) [≅-50%]
Scutellata	957	768 (-19.8%)	434 (-43.5%) [≅-55.5%]
Total	1005	803	458
Colony loss (% lost)	(0%)	202 (-20%)	345 (-43.1%) [≅63%]

There was only a small difference in colony loss between sedentary and migratory beekeepers. Sedentary beekeepers lost 22% of their colonies as oppose the 27% loss of migratory beekeepers (see Appendix). This indicates that the type of beekeeping has little effect on the severity of the problem. Whether a beekeeper moves his colonies or not, if there are Capensis problem bees in his stock the chances of them spreading between colonies are almost equal.

Table 9: Colony lost per region

	NC	FS	SG	NG	NP	MP	NKN	SKN
Total lost	14	54	104	112	50	143	18	57
% lost	8.54	15.79	21.71	43.58	21.01	59.83	6.06	24.15

In some regions the beekeepers reported more colony losses then in others. As table 9 shows this was indeed the case. Beekeepers in the Mpumalanga region lost more than half their stocks (± 60%) followed Northern Gauteng (± 44%).

DISCUSSION

The current problem started with the introduction of *A. m. capensis* colonies in the *A. m. scutellata* area. But unlike previous similar introductions this time the capensis laying workers spread unstoppably throughout the summer rainfall region despite stringent measures to eradicate them. Colonies succumbed to
Cape Problem Bees in all regions. However, the tempo at which apiaries collapsed differed. When beekeepers reported high levels of infestation in all their bee stocks and infestation were found in the colonies that were surveyed, that apiary would be dead within six months without exception. In apiaries where there were only one or two colonies that contained Cape Problem Bees the die-out was slower.

Colonies that lost their queen and where laying workers were present all died within a short period of time, although some seemed to co-exist with the laying workers longer than others.

It is doubtful whether the colonies classified as hybrids by the dissection data were indeed hybrids. Although hybridization was possible, it was very unlikely and the designation “hybrid” rather showed that the classification used was flawed. This assumption is further strengthened when the difference in dissection- and inspection-results is compared. Where the inspection results gave low numbers of capensis colonies, the dissection data gave almost equal numbers for both races. When the colony loss within apiaries are looked at it is clear that colonies and apiaries were quickly lost when laying workers were present. Therefore, if the dissection data were correct, almost all the colonies in all the apiaries should have been dead by the second survey. Contrary to this, the inspection data suggested that some apiaries would be wiped out and in apiaries with low infestation, only some colonies will die-out between surveys. The second scenario was found to be true.

Migratory beekeepers from the northern Gauteng region use to migrate extensively to the *Eucalyptus grandis* plantations for honey production and the Mpumalanga and northern Gauteng region’s beekeepers use to move their colonies into the *Aloe greatheadii* var. *davyana* areas for colony production. Beekeepers has reported much colony loss after utilizing these bee-plants. The results clearly shows that their observations were correct.

However, many beekeepers from southern Gauteng also utilises the Aloe flows and all beekeepers in Kwa-Zulu Natal utilises Eucalyptus flows, and colony loss in these regions were much lower. The author is of the opinion that the forage sources plays a lesser (if any) role than beekeeping practices. The factors that favour infestation i.e. beekeeping practices, reduced queen control, sudden acceleration of brood production, and reduced guarding due to heighten forage activity are present during any nectar- and pollen flow. However, the migratory beekeepers of Mpumalanga and northern Gauteng move their colonies to the same areas, thereby supplying the source of infestation - colonies already taken over - and creating ideal situations for infestation – non-infested colonies in the same area.

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CONCLUSION

There was a misconception amongst South African beekeepers that this country was always without any serious diseases and problems. The truth is that South Africa have had diseases and pests ever since modern beekeeping has been practiced. But we were lucky that the both the Cape- and African honeybee races were hardy and seemed to remain productive even when they were infested or infected.

The Cape Problem Bee was the first condition that really caused large-scale colony loss. The tragedy was that it was preventable, but due to the lackluster attitude of beekeepers prior to the problem no measures were taken to inform novice beekeepers or regulate the industry. Even during the early period of this problem while thousands of colonies died out, beekeepers squabbled over petty things. Small-scale beekeepers blamed professional beekeepers, sedentary beekeepers blamed migratory beekeepers, and some beekeepers ignored the regulations, research results, and suggestions and kept on farming with infested colonies. The problem therefore persisted.

The aim of this study was not to find solutions to the problems but merely to establish the extent and severity of these problems. Both nosema and tracheal mites were more common than what was expected. What influence these organisms have on beekeeping was not established but beekeepers should take note of them and should take preventative measures if they want to optimise their beekeeping operations. On the contrary, the Cape Problem Bee was less of a problem than anticipated. Beekeepers can successfully farm with the problem if they do not create ideal conditions for the easy invasion by laying workers. It seems that certain beekeepers in certain regions perpetuate the problem. Maybe it is time for the Government to enforce the existing legislation and force these beekeepers to kill all their infested colonies.