NOSEMA DISEASE OF THE BUMBLE BEE *BOMBUS TERRESTRIS* (L.).

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CATHERINE ANN McIVOR

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ABSTRACT

A *Nosema*-like microsporidian pathogen of the bumble bee *Bombus terrestris* (provisionally referred to as "NBT") is described in terms of its occurrence, its developmental cycle and morphology.

Variation in *Nosema* infection levels in *B. terrestris* queens from four sites in the central North Island suggested seasonal and climatic effects, with greater infection of queens from milder and/or urban habitats.

Experimental infection of bumble bee workers provided details of the morphology and life cycle of the microsporidian. Features of the somatic stages of NBT observed from stained, smear preparations of tissues were characteristic of the genus *Nosema* (e.g. predominantly binucleate somatic stages comprising meronts, sporonts and sporoblasts). Each group of stages were variable in size and had from 1-4 nuclei. The timing of the appearance of somatic stages was investigated and a life-cycle for NBT was proposed. The minimum generation time of NBT spores at 30°C was approx. 4 days, with 50% of spores appearing within 7 days.

Transmission electron microscopy indicated two spore forms were present, one *Nosema*-like with a polaroplast, diplokaryon, and 14-18 polar filament coils, the other having the same basic features but lacking the polar filament. NBT spores and somatic stages were found primarily in the Malpighian tubules and occasionally in the tissues of the midgut. The remaining tissues were free from infection.

In a cross-infectivity test NBT was found to be slightly infective to *Plutella xylostella*, a Lepidopteran species that feeds on brassica plants. As bumble bees visit brassica flowers it is possible that natural cross-infection of NBT may occur, with other insect species acting as reservoirs for the disease.

Comparisons of the morphology and life history of NBT with other *Nosema* species known from Hymenoptera indicated that NBT was different from all but *N. bombi* Fantham and Porter 1914. It is concluded that the NBT of this study is *N. bombi*.

Experiments were undertaken to examine the transmissibility of NBT and its maintenance in individual bumble bees. Fecal material from naturally and experimentally infected queens was examined and numbers of spores estimated with a haemocytometer. The time taken from the ingestion of heat-killed spores by queens, to their complete elimination in the faeces, was approximately 5 days.

Contamination of the nectar wick with faeces containing NBT spores caused the spore loads of individual bees to increase. Similar results were achieved by

feeding bees directly with faeces (mixed with sugar syrup) containing a known number of spores. No significant reduction in spore numbers was observed from infected bees treated with Fumidil-B (an anti-*Nosema* drug).

NBT infection did not appear to affect the nest initiation or egg-laying behaviour of bumble bee queens nor was there evidence for *Nosema*-linked mortalities.

CHAPTER 1

GENERAL INTRODUCTION

1.1 THE HISTORY OF BUMBLE BEES AND POLLINATION IN NEW ZEALAND

New Zealand has no native bumble bees or honey bees. Honey bees were first brought to New Zealand around 1839 and up to the late 1800's farmers relied on them for crop pollination (Gurr 1961). However there still remained persistent problems with low seed yields from red clover, *Trifolium pratense* L. and lucerne, *Medicago sativa* L.. Darwin (1858) focused attention on the bumble bee as an efficient pollinator of red clover in Europe. On the basis of this knowledge a small number of queen bumble bees were imported into New Zealand in the early 1880's (Nottidge 1890).

Bombus terrestris and *B. ruderatus* were liberated in the South Island of New Zealand in 1885 (Gurr 1957b). Both species survived well and became readily established. In 1906 at least four species were imported and released. Gurr (1964) reported that two of the four became established in the South Island, namely *B.hortorum* (L) and *B.subterraneus* (L) sub sp. *latreillellus* (Kirby). New Zealand now supports four known species of European bumble bees, the most widespread being *B. terrestris*.

Red clover flowers have long corolla tubes and this means only long-tongued insects are able to reach the nectaries (Gurr 1975). However, *B. terrestris* is the only one of the four species that does not have a long tongue, resulting in their tendency to 'rob' the flowers by biting holes at the base of the corolla tube so bypassing contact with the sexual parts of the flowers (Gurr 1975). Honey bees often become 'secondary robbers' by re-using the holes and pollination is further reduced (Prys-Jones & Corbet 1987).

Although *B. terrestris* may not be suited to the pollination of red clover it has since been found to be an efficient pollinator of many other crops (Gurr 1955 , Palmer-Jones & Clinch 1966). Work has been undertaken in Massey University's Botany/Zoology Department, examining the use of *B. terrestris* as a pollinator of kiwifruit and muskmelons (Fisher & Pomeroy 1989a). In Europe, *B. terrestris*

colonies are produced on a commercial level and placed in glass-houses to effect the pollination of tomatoes and other crops. An important feature of bumble bees in general is that they will work more efficiently than honey bees in glass-houses. This is of potential market value where crops, such as tomatoes, are usually pollinated by hand at a considerable cost (in money and manpower) to the grower. *B. terrestris* was chosen as the study species primarily because it is the species used in other studies at Massey University and is abundant within the vicinity of Palmerston North. The renewed interest in the bumble bee as an alternative pollinator (especially in Europe) also implies the need for further research on the problems that are encountered when rearing such an insect on a commercial scale.

The controlled rearing of commercially viable bumble bee colonies is difficult. Even where the bees are able to free forage (outside the laboratory) small nests usually result (Tod 1986). There are many potential problems contributing to poor colony development and some of these, especially social ones, have been studied extensively (Pomeroy 1981, Tod 1986, Van Doorn 1986). However, one aspect that has not really been adequately researched is that of disease.

When bumble bees were introduced into New Zealand from Europe, many species-specific commensals and parasites entered with them. These ranged from a variety of internal pathogens to external mites. At the time of importation no records were kept nor is there at present a comprehensive review of the pathogens affecting bumble bees. Some progress has been made towards compiling a list of those organisms currently found associated with bumble bees in New Zealand (R.P. Macfarlane unpublished manuscript). However, little work has been directed towards gaining a more comprehensive understanding of the impact that these organisms have on the bees as individuals or the colony as a whole. In comparison, diseases of the honey bee *Apis mellifera* have been listed and studied in detail (Bailey 1981) and in particular the protozoan *Nosema apis* Zander which was discovered in 1906. In 1914 Fantham and Porter described a similar protozoan from the bumble bee naming it *Nosema bombi* (after its host *Bombus*). Its status as a new species though is disputed even today but there have been no recent studies reported which resolve this. In the past bumble bees have generally been regarded as "wild" insects and consequently little interest has been shown in their associated pathogens. Now that there has been a resurgence of interest in the bumble bee as a commercial pollinator I believe it is important to investigate the nature of the pathogens affecting them in New Zealand and the degree to which they may be considered detrimental to the health of the bee and its overall productivity. Considering this *N. bombi*

merits further study particularly as there has been no recent comprehensive research done on it. It is important also to determine whether its presence within a colony may produce similar problems as those encountered with *N. apis* in the honey bee and as a consequence hinder attempts at large-scale bumble bee production.

1.2 BUMBLE BEE NATURAL HISTORY

Young queen bumble bees, inseminated during late summer, hibernate through late autumn to spring. In Europe, this may be "anything from 6-8 weeks or even 9 months" (Alford 1975). However, a more temperate climate, as found in New Zealand, reduces the hibernation period to as little as 2-3 months (Cumber 1953a), and in some cases, may eliminate it all together. The queens dig into loose, well-drained soil to a depth of up to 10 cm, and mould a small cavity in which they remain during the hibernation period (Sladen 1912 , Alford 1969). During spring when soil temperatures rise, the queens emerge and spend a few weeks flying, sunning and foraging while their ovaries are still small and undeveloped (Heinrich 1979a , Alford 1975). Once their ovarioles have begun to develop and contain eggs and nurse cells, the queens commence the search for nest sites (Alford 1975,Cumber 1949a).

Typical sites chosen by queens are nests abandoned by small mammals, although this varies with the species of bumble bee and its situation in urban or rural regions. *B. terrestris* queens nest predominantly under ground, although nests have been found in carpet felt, fiberglass Pink Batts (housing insulation), compost heaps, wood piles and under houses in the region of Palmerston North (personal observation). The site chosen usually has material (paper, plant fibre) that can be manipulated by the queen to form a cavity in which she will lay her first eggs. The brood needs to be maintained at a temperature of 28-30 C, so a suitable nest material is important (Hasselrot 1960).

The queen shapes a pollen clump on the floor of the nest and builds on it a cell of wax and pollen. It is in this that she lays her first batch of eggs, with *B. terrestris* queens depositing up to 16 eggs in their first brood batch (Alford 1978). The larvae hatch about 4 days after oviposition and grow rapidly, feeding initially on the pollen below them and later on a pollen/nectar regurgitate from the queen. The larvae remain in a common cell for approx. 10 days and on reaching the fourth instar, spin flimsy, silken cocoons and become separated from one another (Alford 1978). After

10-20 days they each spin a tough, cylindrical cocoon into which they deposit fecal material (meconium). This is incorporated into the wall of the cocoon (Prys-Jones & Corbet 1987). The larvae then pupate.

Workers emerge from 18-26 days (personal observation on *B. terrestris*), after egg laying. In the first 24 hours the young workers (termed callows) do not perform any duties as they are still damp, have soft wings and have not yet gained their distinctive yellow/black colouration (Pomeroy 1977). Eventually the first workers take over all of the foraging and nest duties while the queen remains in the nest. She lays more eggs on top of the cocoons of pupated brood. (Brood rearing is regulated to meet the needs and economy of the colony. The presence of pupal cocoons is usually a signal for the queen to build egg cells and lay eggs (Alford 1978). Empty cocoons are utilised as pollen or nectar storage pots.

The comb develops upward and outward in a somewhat staggered manner, with egg cells formed on the cocoons from each previous laying. Comb shape is often hemispherical, but ultimately depends on the size and shape of the nest site. The worker number increases rapidly (Plowright & Jay 1966) until, at a switch over point males and new queens (collectively known as reproductives) are produced. Once this stage has been reached it is rarely reversed and worker numbers diminish as a consequence.

Once the young queens emerge from their cocoons they stay in the nest for about 5 days after which they embark on a mating flight (Cumber 1953a). Once they have mated they return to the nest and spend time feeding, resting and building up their fat body (necessary for survival during hibernation). Their ovaries remain underdeveloped during this time, only developing slowly during the hibernation period. The young queens then search for sites in which to over-winter and the cycle is repeated.

Males leave the nest when they are 2-4 days old and generally never return. Their primary objectives in the ensuing period are foraging and inseminating young queens (Alford 1975). They die soon after this has been acomplished.

1.3 MICROSPORIDIA IN INSECTS

The members of the order Microsporidia are predominantly obligate,

intracellular pathogens (Kellen 1976). They are generally small (1.5 µm - 20 µm) and are characterised by resting spores which contain a single infective sporoplasm and polar filament. The genus *Nosema* is the largest of all the microsporidian genera and contains approximately 200 species. Most of these are parasitic in insects but representatives occur in almost all the major animal groups and have a world-wide distribution (Sprague 1982).

Microsporidia of the family Nosematidae may be transferred between hosts in two general ways ; by direct contact or indirect contact (Kramer 1976). The former mechanism involves the microsporidian being transferred directly from the original host to a susceptible host. The latter requires the microsporidian spores to be dispersed by physical agents or carried by a vector. Examples of direct contact are a) the transfer of spores from a parent to its offspring during reproduction (transovarial/vertical transmission), b) the consumption of decaying infected cadavers by susceptible hosts, and c) emerging larvae consuming spores adhering to the surface of their egg (horizontal transmission) and d) the injection of spores into the host by infected parasites. Examples of indirect contact are a) mechanical disturbances carrying particles of soil, excreta etc. into the air that may contain clumps of spores and b) predators/scavengers that consume the spore-filled tissues of the host and eventually discharge the unchanged spores with their feces.

There are three possible portals of entry for the microsporidian into the insect host ; the oral, cuticular and ovarial portals (Andreadis 1987). Some microsporidia employ more than one (e.g. *N. heliothidis* from Lepidoptera, Brooks 1973) whereas others (e.g. *N. apis*, Bailey 1963) are restricted to one, commonly the oral portal. Once the microsporidian spore has entered the body of the insect host it undergoes a cycle of development and reproduction. Under the appropriate stimuli (poorly understood in most species) the spore will extrude under pressure a hollow polar filament, the length of which varies between species. The filament is a firm structure capable of penetrating both the peritrophic membrane and the gut epithelium (Weiser 1961). Testing of the peritrophic membrane in the honey bee, by way of dyes, showed that only the large colloidal dye particles (Congo red) were arrested by it (Wigglesworth 1972). However, little ultrastructural work has been done on the peritrophic membranes of the Hymenoptera so it is difficult to know how differentially permeable they may be. When the filament penetrates a cell (of the gut, fat body etc.) a sporoplasm (nuclei and cytoplasm bounded by a membrane) is ejected and this either remains in the cell or becomes amoeboid and migrates to other parts of the body progressively infecting other tissues. The sporoplasm then

undergoes two distinct cycles of growth and division termed merogony and sporogony. This ultimately results in the formation of spores, the number of which varies according to the species. Infected cells containing the spores eventually burst and they are released into the gut, malpighian tubules or body cavity prior to dispersal. These spores are the infective agents in the microsporidian life-cycle, capable of survival in the extra-corporeal environment if necessary before contacting a new, susceptible host.

The infection of individual insects by microsporidia produces physical effects ranging from acute to sub-lethal/chronic to minor/benign. The bacterial flora of the gut determines in part whether a given dose of spores will cause an acute or chronic infection (Weiser 1976). Massive doses of spores can promote either acute infections or septicemias without any development of the microsporidian. Bacteria entering the wounds (made by filaments) in the gut wall can instigate a septicemia in the host prior to the incubation period of the microsporidian (Weiser 1976). Acute infection (and septicemias) result in the premature death of the insect and tend to limit spread of the disease as the spores remain in the cadavers. Sub-lethal or chronic infection is the most common form and may result in a loss of vigor, reduced fecundity, mating success and overall longevity in the insect (e.g. *N. carpocapsae*; Mercer 1981). It also leads to the spread of infection through the horizontal (between host generations) and/or vertical transmission (within host generations) of spores. Minor or benign infection may not produce any obvious symptoms, the insect surviving and reproducing as well as a non-infected individual. If the physiological functions of the host are not seriously damaged by the microsporidian the insect will often survive the infection. This has been indicated in nosematosis of the honey bee - the mid-gut epithelium has great regenerative powers and a complete recovery can follow an infection originally localised in the gut (Veber & Jasic 1961).

Many factors can influence the overall effects that the microsporidian has on its insect host. The development of the protozoan in the insect can be restricted by the tissue it infects, the infective dose the insect originally ingested and the instar of the insect affected (Weiser 1976). The physiological state of the insect at the time of infection will also have an effect (e.g. if it is a female with brood). Stressors, when considered as inadequate food, abnormal temperatures and humidity, can influence infected insects by shortening the duration of the infection and emphasising the inability of the infected individual to adapt to the change in conditions (Weiser 1963).

1.3.1 Control measures

The introduction of various insect antibiotics in recent years has helped to reduce much of the effort required to maintain disease-free groups of insects. Perhaps the most detailed studies have been on the containment of *N. apis* of the honey bee. Experimental work incorporating various antibiotics (including Urotropin, Apimycin, Nosematose and derivatives of fumigillin) have highlighted the potential value of fumigillin as a treatment for *N. apis*. Fumigillin has also been applied as a general control for many other microsporidian parasites (Lewis & Lynch 1970).

Fumigillin is an antibiotic with amoebicidal properties (McCowan et al 1951). Early trials testing the drug produced striking results in the honey bee, substantially reducing numbers of nosema-infected individuals. Bailey (1953) determined that the fumigillin acted on the microsporidians mainly by arresting or destroying the vegetative stages. Later studies, (Hartwig 1971) indicated that the antibiotic appeared to act at the DNA level by inhibiting the DNA replication by the parasite but without actually affecting the DNA replication within the cells of the host. An added benefit proved to be the restoration of RNA synthesis in the host cell after treatment with fumigillin.

A readily available form of fumigillin is Fumidil-B (Abbott Laboratories, Chicago) and this is a preparation used extensively by apiarists. The antibiotic was used over the course of the study in an effort to maintain disease-free bees and/or reduce their susceptibility to infection. This was based on the positive effects of treatment as seen in the honey bee (Kunst & Tomasec 1965, Furgala & Maunder, 1978) and also on the successful treatment of queen bumble bees with Fumidil-B (De Jonghe 1986).

1.4 SOCIAL INSECTS

Studies on insect-associated microsporidia are often directed towards a) gaining an understanding of the pathogen and its relationship with the insect and/or b) the possible application of the microsporidian as a biological insecticide. These are the approaches used where the study insect is considered a noxious pest. In direct comparison, studies on commercially valuable insects (e.g. *Bombyx mori, Apis*

mellifera) are undertaken to determine a measure of control for the pathogen itself in order to maintain healthy, disease-free insect populations. When investigating the microsporidians that affect social insects any of the three study types may be implemented. It is important to realise that a general understanding of pathogens (in terms of pathogenicity, transmission etc.), as developed through studies on other insect groups, will include features not directly applicable to social insects and their pathogens. Various characteristics of the insect society, such as divison of labour, rearing of the young and cleaning the colony, all influence the level of infection and transmission of the pathogen within the population (Maddox 1987). Because the colony resembles in many ways an individual as well being comprised of many inter-related parts, studies can be done between or within colonies as a consequence.

Some of the insects on which work has been done include ants, termites, honey bees and bumble bees. Within these groups microsporidian infection may range from chronic to benign, attack a variety of body tissues and be transmitted ovarially or orally.

ANTS : At least seven different species of microsporidian have been described from ants. One of these, *Thelohonia solenopsae* of the fire ant (*Solenopsis*) affects the ovaries of the queens and the fat tissue of workers and sexuals. It is not transmitted orally implying a probable transovarial route. It tends to produce chronic pathogenic effects, reducing the longevity of the infected colony (Knell et al 1977). Few long-term studies on these microsporidia have been undertaken but it is believed that infection levels vary from one season to another.

TERMITES : Very little is known regarding the microsporidian parasites of the *Isoptera*. Kalavati (1976) added four new species to the small list of three previously known. One of these, *Gurleya spraguei*, was found to infect the termite *Macrotermes estherae*, invading the fat tissue and consequently was not transmitted via fecal material. Knowledge of the pathology of the microsporidian is poor but it is believed that the social activity of the colony results in seasonal fluctuations in prevalence.

HONEY BEE : Of all the social insects the honey bee has recieved the most research attention. Many books, reviews and scientific papers have been published on the honey bee *Apis mellifera* and its associated pathogens and commensals, a large proportion of which are devoted to the parasitic protozoan *Nosema apis* Zander. First described in 1906, *N. apis* was found to primarily affect the midgut epithelium of the adult honey bee and was occasionally found in the Malpighian tubules. Claims have been made concerning other sites of infection (Steche 1960 ,

Sokolov & Grobov 1963), however in most cases they have not been substantiated (Bailey 1981). Generally, infected bees show no outward symptoms and even heavily infected bees are seen to behave normally. The larvae are not susceptible to infection and only horizontal transmission is known. Worker bees are the primary hosts although drones and to a lesser degree queens, may become infected. It is the infection of the queen that leads to the decline of the colony. This is effected by damage incurred to her ovaries resulting in the failure of eggs to hatch, eventual cessation of oviposition and ultimately her supersedure or death (Hassanein 1951). Infected bees generally live only half as long as healthy bees and afflicted colonies are weakened, although they are rarely decimated by the disease.

Nosema infection disrupts the age-duty sequence and upsets the division of labour of workers within the colony. The infected workers appear to be physiologically older than healthy bees of the same age and perform various duties earlier as a consequence (Wang & Moeller 1970). There is also a correlation between infection with *Nosema* and brood rearing. The stress on the metabolism of workers caused by brood rearing leads to conditions that favour the development of the disease (Butler 1976). These changes within the individual bee also affect the colony as a whole and result in an overall increase in *Nosema* levels.

A notable feature of *N. apis* is the consistent seasonal variation with infection levels highest in the spring and declining over the summer (Weiser 1961). Various explanations for this have been suggested, especially the idea that the bees during winter are more likely to defecate on the comb and healthy workers cleaning the combs in early spring (in preparation for brood-rearing) may become infected if spores are present (Bailey 1955).

N. apis has a global distribution with levels of infection varying with geographical distribution (Weiser 1961, Bailey 1981). In New Zealand, *N. apis* is a significant problem in the maintenance of honey bees and it is estimated that nearly all apiaries are currently infected at some level (L. Malone pers. comm.).

Of all the insect-pathogen associations the protozoan diseases of social insects are perhaps the most complex. Basically, it is the social behaviour of the insect colony as a whole that (probably more than any other factor) that influences infection levels, the development of seasonal fluctuations and epizootics.

1.5 REVIEW OF LITERATURE ON *NOSEMA* IN BUMBLE BEES

Throughout studies on microsporidia there has been one recurring problem - the lack of a universally acceptable classification. Today, perhaps the most comprehensive taxonomic studies on the microsporidia are those proposed almost simultaneously by Sprague (1977) and Weiser (1977). Both have similarities at the higher taxonomic level but many differences are apparent especially at the genera and family levels. Sprague's system, updated in 1982, is the more widely used. As advanced technologies (e.g. SEM and TEM) reveal new information on microsporidian morphology the old bases for classification become redundant (Larsson 1986). These include classifications based on spore shape and size, site of infection in host tissues and host range. The terminology used to describe life-cycle stadia has also altered with time often making comparisions of studies on various microsporidian species difficult (Jacobs 1976). In the case of *Nosema bombi*, first described in 1914 by Fantham & Porter, the above mentioned problems apply. Only the light microscope was utilised with the taxonomic status determined partially on the host species (*Bombus*) and on features of the life-cycle and spore size and shape. These facts alone give cause for questioning the true identity of *N. bombi* and this emphasised by the number of conflicting studies concerning *N. bombi* since 1914.

Research on the microsporidia affecting bumble bees has been limited. Early studies on *N. apis* (Fantham & Porter, 1913) indicated that it was possible to infect a number of species of *Bombus*, including *B. terrestris* L., with the microsporidian. In the following year Fantham & Porter identified a new species of *Nosema* which they named *N. bombi* after its host *Bombus*. They described it as being distinctly different from *N. apis* in terms of spore morphology and tissue specificity (features no longer considered suitable for taxonomic purposes). This may explain why the few studies concerning nosema disease of the bumble bee are mostly contradictory and cast doubt on the true status of *N. bombi*.

Many authors claimed to have succeeded in cross-infecting bumble bee species and the honey bee *A. mellifera* with *N. apis* and *N. bombi* respectively (Fantham & Porter 1913 , Kudo 1924, Showers et al. 1967). Yet others argue that the bumble bee is not susceptible to infection by *N. apis* (Uspenski 1949, Weiser 1961). Showers et al. (1967) ventured the opinion that in the case of some studies where there was difficulty in accurately identifying the nosema (e.g. Skou et al. 1963), the infection was probably caused by *N. apis* and not *N. bombi* as stated. Weiser (1961) believed that the morphological differences between the two nosemas were not

sufficient to warrant claiming *N. bombi* as a new species and implied that the two were probably synonymous. This opinion is held by various authors (Showers et al. 1967, R.P. Macfarlane unpublished manuscript) and is reinforced by evidence that not only are microsporidia are often cross-infective between hosts but the mophology of the parasite may actually be altered in different hosts (Canning 1977).

Skou et al. (1963) noted that the seasonal variation of nosematose in the bumble bee appeared similar to that seen in the honey bee. However, the seasonal variation idea is perhaps not valid for the bumble bee as the colony is annual and not perennial like the honey bee. For the same reason it is perhaps uninformative to make direct comparisons with honey bee colonies that show high levels of nosema infection during late winter and early spring. The observation that *Nosema* incidence increases (in both the honey bee and the bumble bee) with a decrease in the ambient temperature is perhaps more important (Showers et al. 1967). Although the bumble bee as a colony lasts only a few months it is the new queens produced during that period that overwinter, emerging the following spring to reproduce. The bumble bee queens that are infected with *Nosema* during the summer are more likely to die prior to or during hibernation as indicated in the study by Skou et al. (1963) although many infected queens do survive to emerge in the spring (Betts 1920, personal observation on *B. terrestris*). The bumble bee queen appears to be the one individual that maintains the infection within the bumble bee population from season to season.

Other than the studies mentioned above, little or no work has been done on elucidating the exact nature of *N. bombi* and whether it can be classified as distinct from *N. apis*. Most of the studies have concentrated on cross-infection between the honey bee and the bumble bee the results of which, even today, have not been agreed on. The productivity of *Nosema* infected colonies has been examined (Fisher & Pomeroy 1989b) but the overall effect of the microsporidian on individuals not been studied. As large, healthy colonies of bees are needed for their commercial application, any factor(s) that may reduce colony productivity or longevity will need to be identified.

A study of *B. terrestris* and one of its pathogens has presented a chance to elucidate some of these problems. The pathogen, a microsporidian of the genus *Nosema*, is referred to from here as "NBT" (= *Nosema Bombus terrestris*) after the convention of Watanabe (1976).

1.6 OBJECTIVES OF STUDY AND THESIS PLAN

This project describes a microsporidian observed from the bumble bee *Bombus terrestris*. Firstly its distribution, incidence and taxonomic status are determined and then aspects of the pathogen/host interaction are examined.

Chapter 3 examines the distribution of the pathogen and its frequency in the populations sampled.

Chapter 4 describes the pathogen in terms of its life cycle and spore morphology. Comparisons to other microsporidia are made and its taxonomic status determined.

Chapter 5 examines the relationship between the pathogen and its host in terms of its infectivity and transmissibility.