EFFECT OF ETHYLENE DIBROMIDE ON THE CONTROL OF WHITE GRUBS AND ITS IMPACT ON *CORDYCEPS* IN SOILS OF THE TPC SUGARCANE ESTATE MOSHI, TANZANIA

BY

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ABSTRACT

The populations of white grub pest of sugarcane, and that of *Cordyceps*, a naturally occurring fungal pathogen of white grubs, were determined in the four fields of the TPC sugarcane estate. The effects of ethylene dibromide (EDB), a commercially used soil fumigant, was assessed in controlling the white grubs and its impact on *Cordyceps*. Also the contribution of *Cordyceps* as the biocontrol agent of white grubs was evaluated both in fields and in the laboratory. White grubs populations (in the four fields) varied significantly among four selected fields with grub densities varying from 25 000 to 93 000 grubs per hectare. These variations were possibly due to differences in field location and soil types and also the number of crop ratoons. Similarly the populations of *Cordyceps* differed significantly between fields ranging from 600 to 4000 *Cordyceps* clavae per hectare. The variations were attributed by initial population of white grubs, and soil properties. Ethylene dibromide significantly reduced the populations of both white grubs and *Cordyceps* in the treated plots, with maximum impacts at the third week after EDB application. In the laboratory, reduction in the population of white grubs by EDB was consistent to the sliding scale of concentration. Although EDB reduced significantly the germination of the *Cordyceps* clavae from infected cadavers, its effect on fungal growth and the branching of germinated clavae were not significant. Although estimated mortality of white grubs caused by *Cordyceps* in stress rearing experiment was low (0.27-27%), from field surveys the contribution of the fungus as a natural control agent of white grubs was very high (54-94%). Studies on the effect of EDB on non target organisms

in sugarcane fields, and development of alternative white grubs control strategies which are sustainable and less harmful to the environment have been recommended.

DECLARATION

I, JUMA TUWELANGO KAPAMA, do declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and it has not been submitted for a higher degree award in any other University.

Signature..... 

JUMA TUWELANGO KAPAMA

Date: 30th May 2001

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This dissertation is dedicated to my parents Othman and Mariam, my wife Neema and our children Othman and Mariam, who through their participatory contributions I got my education.

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LIST OF ACRONYMS

BHC	Benzene hexa chloride
CEC	Cation Exchange Capacity
DDT	Dichloro diphenyl ethane
Ece	Electrical Conductivity of the Saturated Extract
EDB	Ethylene Dibromide
LS	Loamy sand
NORAD	Norwegian Agency for Development
OC	Organic Carbon
SL	Sandy Loam
SUA	Sokoine University of Agriculture
SRI	Sugarcane Research Institute
TCH	Tons of Cane per Hectare
TCHM	Tons of Cane per Hectare per Month
TPC	Tanganyika Planting Company

CHAPTER ONE

INTRODUCTION

Sugar is a commodity of great importance in the world (Senkoro, 1988). Approximately 60% of all the sugar produced on the global scale comes from sugarcane (*Saccharum officinarum*), a tropical monocotyledon plant characterised by high sucrose content in its mature stalk (Lakshmikarintham, 1983). Other sources of sugar include sugar beet, sugar maple, sorghum and few types of palms.

The world sugar production during the 1992/93 market year stood at 116.2 million metric tons while world sugar consumption was 112.4 million metric tons, leaving 24.6 million tons (21.9%) as world surplus sugar stock. Tanzania produces on the average 129 800 tons of sugar, which is less than half of the country's demand of 400 000 tons [Ministry of agriculture (MOA)], 1989.

The Tanganyika Planting Company (TPC) in Kilimanjaro region is one of the major sugar producing estates in Tanzania. Other sugar estates in the country are Kilombero and Mtibwa in Morogoro region, Kagera in Kagera region, and Mahonda estate in Zanzibar. Sugarcane production at the TPC estate is affected by several factors including drought, low soil fertility, and pests. Of all the pests, insects, especially the soil borne ones, are a major factor which reduces sugarcane production. White grubs are the major pest of sugarcane at the TPC (Katundu, 1999), but not in the other

estates although they do occur (e.g. at Mtibwa). White grubs are root-eating larvae of beetles, the principal species at TPC estate being the red cane beetle *Cochliotis melolonthoides* Gerst (Coleoptera: Scarambaeidae) (Jepson, 1956). The larvae of this beetle exert profound yield losses of sugarcane which are currently estimated to be in the range of 25-30% annually [Sugarcane Research Institute (SRI), 1995]. Because of their root-eating habit, they exert a high degree of injury to sugarcane roots (Mohyddin, 1996), which has resulted in loss (death) of the crop in some severely affected fields at TPC. The ability of the grubs to damage the underground portion of the crop, coupled with their complicated and overlapping life cycles, has made the cane grubs the most complex sugarcane pest for a long time at the estate.

The first record of the melolonthid larvae as a pest of sugarcane at TPC dates back to April 1941, when about 25 acres of the estate were found infested (Jepson, 1956). Currently, over 30% of the 5461 ha under sugarcane are highly infested by the white grubs (Mkodo, 1998). Since then, three methods of controlling white grubs at TPC have been used namely, cultural, biological and chemical means (Jepson, 1956). Chemical control has been practically confined to soil treatment for larvae control (Jepson, 1956). The list of chemicals used over time is given in Appendix 1. Soil fumigation with ethylene dibromide (EDB), which commenced in 1984, has proved relatively economical and possibly the most effective and quick method of controlling white grubs (Maloy, 1993). At TPC, all the fields with grubs count equal to or above the established estate threshold level of 50 000 grubs per hectare at harvest are fumigated with EDB 45%, before the next planting.

For all fields with grub numbers above 100 000 grubs per hectare at harvest, the sugarcane is uprooted and the soils are fumigated until the grub densities are at or below the 50 000 threshold. The rate of EDB has increased from 60 l/ha in 1984 to 165 l/ha at present. However, the efficacy of EDB in reducing the grub densities has been decreasing since the 1989/90 cropping season (Mkodo, 1998). Although the storage, application methods, application rates, and quality specifications of the EDB used have remained the same, the efficacy of EDB has shown to decrease and reason for this is yet to be established.

Like all other soil fumigants, EDB is applied in invariably large doses. Currently, EDB application rates at TPC range between 100 and 165 litres/ha (TPC Limited, 1997), depending on the level of infestation in a given field. Higher doses are known to have broad spectrum effects which go beyond the target species. Among the non target soil inhabitants are beneficial macro and microorganisms. Several natural enemies of white grubs are found in the soil, including entomopathogenic fungi (fungi infecting living insects) of the genera *Metarhizium, Beauveria*, and *Cordyceps* (Lomer, 1996) *Cordyceps* is so far the main natural enemy of the white grubs at TPC. *Cordyceps* belongs to the family of Clavicepitaceae of the order Clavicipitales in the subgroup Ascomycotina (Lomer, 1996). This fungal pathogen has been found to parasitise and kills the third instar larvae of the white grubs (Smith, 1997; Hocking, 1968). However, it is possible that EDB used in large doses has adverse effects on *Cordyceps*, thus undermining its advantage as a natural enemy of white grubs.

The purpose of this study was to evaluate the effectiveness of EDB for control of white grubs and its impact on natural populations of *Cordyceps*.

The specific objectives of the study were:-

1. To evaluate the effects of EDB on control of white grubs as the target pest species of sugarcane,
2. To determine the populations dynamics of *Cordyceps* a natural occurring pathogen of white grubs in sugarcane fields of the TPC estate,
3. To investigate the contribution of *Cordyceps* in the control of white grubs,
4. To investigate the effects of EDB on *Cordyceps barnesii* populations, growth and development in the sugarcane fields of the TPC estate,

CHAPTER TWO

LITERATURE REVIEW

2.1 The white grub problem at TPC estate.

White grubs have been a pest of sugarcane at TPC since the crop was first grown in the early 1930s (Jepson, 1956). The majority of the larval populations of white grubs that causes economic losses and found in the soils of the TPC estate are those of *Cochliotis melolonthoides*. The first record of *C. melolonthoides* as a pest at TPC, inflicting heavy damage, was in 1941 (Katundu, 1999). *Cochliotis melolonthoides* has continued to invade new sugarcane fields, and the most extensive infestation has been along the Ruvu river in the western part of the estate. By late 1970s, the pest had already advanced more than 10 km along the river, covering more than 30% of the area under cane (Mkodo, 1998). Currently, more than 50% of the area under cane is infested with varying degrees of population intensities of the pest (Katundu, 1999). The economic importance of this pest has been discussed in section 2.3.

2.2 Damage on sugarcane by white grubs

White grubs are highly poliphagous insects with a wide adaptability to different host plants, including sugarcane. Their destruction of the roots and underground portion of the stalk causes stunting, wilting and yellowing of cane leaves. This is followed by drying up and eventually death of the sugarcane roots (Jepson, 1956). The affected stalk also lodges and the stool can easily be pulled out. Normally, it is the third instar larvae that causes the greatest damage. Severe symptoms occur when the crop is under moisture stress and when the pest populations are high.

2.3 Economic importance of white grubs at the TPC estate

It has been estimated that one tone of sugar per ha is lost due to grub infestation (Katundu, 1999). This is equivalent to the amount of sugar obtained from a harvested area of 300 ha. In monetary terms at current price, this loss is approximated at TShs. one billion. In addition, millions of shillings have been spent in the purchase and application of insecticides against the grubs (Katundu, 1999). There are other sources of economic losses, the most important one being the reduction in number of profitable ratoons on which the prosperity of the estate depends, together with additional costs such as paying for labour in gap. filling , ploughing, replanting infested fields and fertiliser application (Jepson, 1956). Therefore, without strategies to control the grubs, the economic losses due to this pest are enormous.

2.4 Use of chemicals against white grubs at the TPC estate

The history of chemical control of white grubs at the TPC estate is as old as the histories of the pest and cane crop (Minja *et al.*, 1993). A variety of chemicals ranging from those with high knock down effect, to those with high persistence to the environment, have been in use since the pest was recognised over fifty years ago.

Though the history of soil fumigation goes back as far as 1896, EDB as soil fumigant came into use world-wide in 1947, and has become a popular chemical to control soil borne pests (Maloy, 1993). It was commercially adopted as a control measure of white grubs at TPC in 1984 (Mkodo, 1998). The chemical is applied in the soils between the cane rows of grub infested fields under pressure, 15-20 cm beneath the surface of soil. For the past ten years, about 1 529 100 litres of EDB have been applied at the TPC estate (Appendix 2), and yield trends resulting in from the usage of EDB are shown in Appendix 3. The declining efficacy of EDB as control measure of white grubs at the TPC estate is illustrated in Appendix 4, and possible reasons could be the development of resistance against EDB among the members of the *Cochliotis* sp. As efficacy of EDB declines it is high time to explore other ways to control the white grubs, including biological control methods.

2.5 Toxicity and persistence of EDB in soil

The dynamics of EDB, like all other soil fumigants in soils, are influenced by various factors and processes operating in the soil system, and also by its physico-chemical and biological properties. The soil factors (soil texture, soil structure/porosity, soil moisture, soil temperature, soil organic matter and plant residues) are the ones that determine the effects of EDB as a fumigant on living systems across different soils.

The toxicity of EDB in soils is well documented by other authors (Maloy, 1993; Israel Bromo Compounds Limited. 1973). Generally, phytotoxic properties and plant injury of EDB occur if treated soil is not purged of the residual fumigant. Also, toxic residues may be left behind when the fumigant decomposes. EDB is a halogenated hydrocarbon and some breakdown products, especially bromine, are injurious to sensitive plants such as carnation, onion, and sugar beet (Maloy, 1993). The decomposition is mediated mainly by microbial activities. EDB in soils alters availability of some plant nutrients by killing microorganisms, thereby stopping their activities, which would result in nutrient release (Maloy, 1993). The nitrogen flush (Maloy, 1993) is the usual explanation of improved plant growth after fumigation when no pathogens or other pests can be associated with a plant problem. However, EDB has not been reported to exert in soils a detrimental effect to plants, which is associated with elimination of endotrophic mycorrhizae or fungi.

Ethylene dibromide has a very low vapour pressure [about a third that of water, but fairly high solubility in water (Maloy, 1993)]. The fact that the chemical moves mainly in soil water rather than through air spaces in soils has at times resulted in groundwater contamination (Maloy, 1993). The EDB persists in soil, in the active mode, to a maximum of three weeks, depending on climate and soil physical conditions. Since EDB solidifies at 9.2-9.7 °C, and it is usually gaseous above 40 °C (Maloy, 1993), it is ineffective in soils of low temperatures. Therefore it is used almost exclusively in warm climates. Its mode of action is narcotic (Hurst, 1978). Because of its solubility in lipids, it dissolves in the fatty tissues around the nerves and induces a narcosis which is normally reversible (Hurst, 1978). This could be another reason why EDB is not so effective in the control of white grubs.

Differences in persistence of pesticides in different types of soils are well documented (Mayer *et al.*, 1990; Royuela *et al.*, 1990; Loux and Rees, 1992; Pusino *et al.*, 1992). Although little has been documented on persistence of EDB in soils, the contribution of organic matter to the total adsorption has been observed (Maloy, 1993). Adsorption was high in soils with high organic matter content. A sequential loss in adsorptive capacity was observed when the soils were treated with hydrogen peroxide to oxidise the organic matter. Coarse textured soils generally are easy to fumigate while fine textured soils like clay and clay loams are more difficult, the clay fraction adsorbs a relatively large amount of fumigant up to about 10% of its own weight while organic matter adsorbs even larger amounts (Maloy, 1993).

Though data on the effect of soil pH on EDB are scarce, the pH of the soil has been reported to affect pesticide persistence in the soil. Maximum adsorption of s-triazine occurred in the vicinity of their pKa values (Weber, 1970). As the pH decreased, the herbicide molecules became protonated and, therefore, more cationic, thus becoming more strongly attracted to soil colloids.

EDB with the acidity of less than 10 ppm (Israel Bromo Compounds Limited, 1973), is undissociated molecule and it does well on the soils with low pH values (below 7), than at high pH and because they are neutral molecules they easily enter the cells of organism and show toxic action faster than do the ions (Cochrane, 1958).

The soil environment into which pesticides are introduced determines to a large extent the rate of their disappearance, and differences in persistence in a particular soil type can be attributed to variations in soil temperature and moisture levels. Fumigant move best in moist soils with water potential between -6 and -15 bars and the best results are usually obtained in the range of 10 to 27 ^{0}C (Maloy, 1993).

Weather and climate are among other factors that influence the rate of disappearance of pesticides through their effect on volatilisation, surface removal in run off, and downward movement in the soil profile. For example, DDT and alachlor have been reported to dissipate very fast in soils of warm moist climates than in soils of cold dry climates (Samuel *et al.*, 1989; Walker *et al.*, 1992).

Weather and climate alter persistence through their effect on degradation (photo-chemical, biological) and non-biological loss of pesticide, detoxification, increased toxicity, or conversion of one group of pesticide to another (Alexander, 1985). These factors may contribute to pest tolerance or resistance to a pesticide. Pesticide formulations, and method of pesticide placement in the soil, are also among the factors that determine pesticide persistence in soils (Talbert and Fletchall, 1964). There is a general agreement that pesticide residues can affect plant growth. Conversely, the presence of plants can significantly affect pesticide upon their decomposition and become humus (Maloy, 1993). Organic matter can adsorb large quantities of fumigant, and it may require up to 10 times as much EDB for effective fumigation when plant residues are added to the soils (Maloy, 1993). However, residues such as wheat straw, corn stalks, potato haulms can also cause rapid loss of fumigants from soil by creating channels (chimneys) to the soil surface (Maloy, 1993). So for effective fumigation using EDB in sugarcane all crop residues should be well incorporated into soil by rototilling or disking.

2.6 Entomogenous fungi : The genus *Cordyceps*

Entomogenous fungi (fungi infecting living insects) have aroused interest as a possible biological means of controlling insect pests (Robinson, 1967). In nature they are reported to cause a regular and tremendous mortality of many pests in many parts of the world. They are known to constitute an efficient and extremely important natural control of insect pests (Robert *et al.*, 1988). Such fungi are particularly

effective on some insect larval stage when the cuticle of the host is comparatively soft (Robinson, 1967). Their capacity for rapid hyphal development can cause severe damage within an insect larva.

All the species of the genus *Cordyceps* grow on insects, and the types affected are ranked in the following order: Hemiptera, Diptera, Lepidoptera, Hymenoptera and Coleoptera (Massee, 1895). The genus consists of an erect stem-like sterile portion composed of a fascicle of irregularly parallel septete hyphae, which are white internally, but tinged with colour externally. In many species, *Cordyceps* gives off numerous short lateral branches forming the minutely velvety or downy exterior of the stem. Different species can parasitise different stages of insect development. The species of *Cordyceps* are regarded as being parasitic because it is known in several instances that the fungus attacks the insect while it is still alive, and the fruiting stage of the fungus usually develops after the death of the host (Massee, 1895).

One of the first comprehensive reviews of this genus was that given by Massee (1895). Since then, many other accounts have been published on different species (Robert *et al.*, 1988; Evans *et al.*, 1987 and Robinson, 1967). The genus is cosmopolitan, being best represented in temperate regions with 27 species occurring in Old World, while New World records 29 species (Massee, 1895). Africa presently registers only two species (Massee, 1895), which are *Cordyceps militaris* Link and *Cordyceps barnesii* Thwaites. One of the species, *Cordyceps barnesii*, is found in

Tanzania and is well documented both at Mtibwa (Morogoro region) and TPC sugarcane estates (Smith, 1997; Katundu, 1999).

2.6.1 *Cordyceps barnesii*: the white grub pathogen at TPC

2.6.1.1 General

Cordyceps barnesii Thwaites is parasitic upon the larvae of the lamellicorn beetle (belonging to the Melolonthidae, and an old term for Scarabids). The fungus is common in soil-borne insects. At TPC it is very common in sites with a previous record of high population densities of the host insects, *Cochliotis melolonthoides* Gerst.

2.6.1.2 Occurrence of *Cordyceps barnesii*

There are few references in the literature regarding the occurrence, distribution and economic importance of *Cordyceps barnesii*. *Cordyceps barnesii* was first described in Sri Lanka on a Melolontha, a pest of coffee (Evans *et al.*, 1999). The species name *barnesii*, was assigned to it by Thwaites after his friend H. E. Barnes who first directed Thwaites' attention to it (Massee, 1895). It was concluded that this scarabid pathogen was confined to the eastern hemisphere. *Cordyceps barnesii* is a specific parasite of *Cochliotis melolonthoides* which is native to Tanzania (Jepson, 1956). Little is known about the distribution of the species to other parts of the world because it has not been studied much. In sugarcane fields at TPC, the species appears

to be an important mortality factor of the third instar of *Cochliotis* larvae (Katundu, 1999). *Cordyceps barnesii* has the potential of becoming an important fungus for use in augmentative biological control of sugarcane white grubs (*Cochliotis melolonthoides* Gerst).

2.6.1.3 Life cycle of *Cochliotis melolonthoides*

Cochliotis melolonthoides has a life cycle of one year with the essential stages as follows. The adult beetles usually emerge from the soil after the onset of the long rains in mid March, as well as early October during the short rains. They mate and return to shelter under trash on the soil surfaces. The females lay eggs between 20 and 100 over a period of several months. The period between egg laying and hatching of the larvae is about fifteen days. Eggs and young are particularly abundant in soils from December onwards, with 80 per cent of the first and second instar larvae appearing in April (Jepson, 1956). The grubs pass through three stages, the first two stages or instars lasting for about three weeks each. The third instar larvae involve a growth period of 4-5 months. They are most abundant from June to August. Finally they make their way deeper in the soil where they make cells ready for the resting period of pupation. After a pupal period of about fourteen weeks, varying with soil moisture, the adults emerge *en masse* in early October and fly for a very short period at dusk though do not cover any great distance before coming again in the soil (Jepson, 1965).

2.6.1.4 Biological control of white grubs by *C. barnesii* : Intervention of *C. barnesii* in the life cycle of *C. melolonthoides*

Cordyceps barnesii seems to infect only the third instar larvae of *C. melolonthoides* in the 10 cm top soil (Evans *et al.*, 1999). Hocking (1966) concluded that there must be a very remarkable change in behaviour of the infected larvae and such changes are poorly known and remains subject of considerable speculation.

The possible mechanism of attack of white grubs by *Cordyceps* is by contact with spore contaminated soils. through external cuticle. Since the larval stage of *C. melolonthoides* is confined to the terrestrial habitat, the chances of the larvae to contaminated by the spores of *Cordyceps* are very high. Spores germinate on the host cuticle and hyphae penetrate inside the host using enzymes and mechanical pressure (Lomer, 1996).Inside the haemocoel the fungus proliferates rapidly by budding or hyphal fission and the resulting cells (blasto spores) spread out through the body. Death of the host is caused by extensive mycelial colonisation, causing asphyxiation or starvation or by toxins released in the yeast phase. The fungus ramifies throughout the inside of the larvae, and eventually death occurs, when infected grubs attain the curled and flattened abdomen and in-bent legs of the pre-pupal stage (Evans *et al., 1999*). The cadaver desiccates as the hyphae use host derived nutrients and water for further development (Lomer, 1996).

Host death marks the end of a parasitic phase of fungal development. The mycelium then grows saprophytically, producing antibiotics against intestinal bacterial flora. In the absence of favourable conditions the fungus survive dry periods in these mummified host larvae. When the environmental conditions are favourable, the fungus grows outwards and produce a *Cordyceps* clava. Under favourable climatic conditions most *Cordyceps* are able to produce resting propagules (spore), which allow the fungus to overseason or to withstand adverse conditions in the absence of the host (Robert *et al.*, 1988). It is probable that these spores germinate in periods coinciding with the first instar emergence, showing the perfect synchrony with the host life cycle (Robert *et al.*, 1988).

The potential impact of the fungus on the scarabid pest populations appears to effect good control of the grubs. One limitation is the slow natural spreading of the fungus and can kill vast number of grubs, but pest populations are never reduced below economic threshold levels (Evans *et al.*, 1999). For example Minja *et al.*, (1993) recorded only an average of 3.5% natural infection at TPC, and in Indonesia natural infection of between 10 and 25% with infection depending on soil type and moisture conditions (Smith, 1997).

Factors which may retard the rate of infection include the degree to which the habitat is exploited due to the disappearance of the specific hosts or the loss of optimum conditions for infection or a combination of both. Also environmental conditions

especially relative humidity or rainfall, amount of available host, early availability of host and amount of pathogen inoculum in the soil (Robert *et al.*, 1988).

To improve the infection rate, and possibly minimise the environmental impact, the estate field management could imitate the example from Indonesia where sugarcane workers actively distribute/spread infected larvae around the fields to increase the infection levels of white grubs by *Cordyceps* (Evans *et al.*, 1999). This will be economical if *C. barnesii* is to be regarded as a useful control agent.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

3.1.1 Location

TPC estate is located in the Arusha Chini area in Moshi-rural district, in Kilimanjaro region (Figure 1), with a gross area of about 6000 ha of which 5461 are under cane cultivation (Mkodo, 1998). The estate is located 20 km South of Moshi town, at an altitude of 700 m above sea level, the grid reference 3° 32"S and 37° 20"E. The area is bordered by villages which include Pasua and Matindigani to the north, Mabogini to the north-east, Mtakuja and Mserekia to the east, Samanga on the south and Msitu wa Tembo, Londoto, Rundugai and Kikavu Chini to the west (Figure 2).

3.1.2 Vegetation and Land Use

The natural vegetation of Arusha Chini is an open woodland savanna with some areas covered with shrubs, and soda grass (*Digitaria abyssinica*). Towards the south, the vegetation changes more and more from savanna bush into a typical salt-bush.

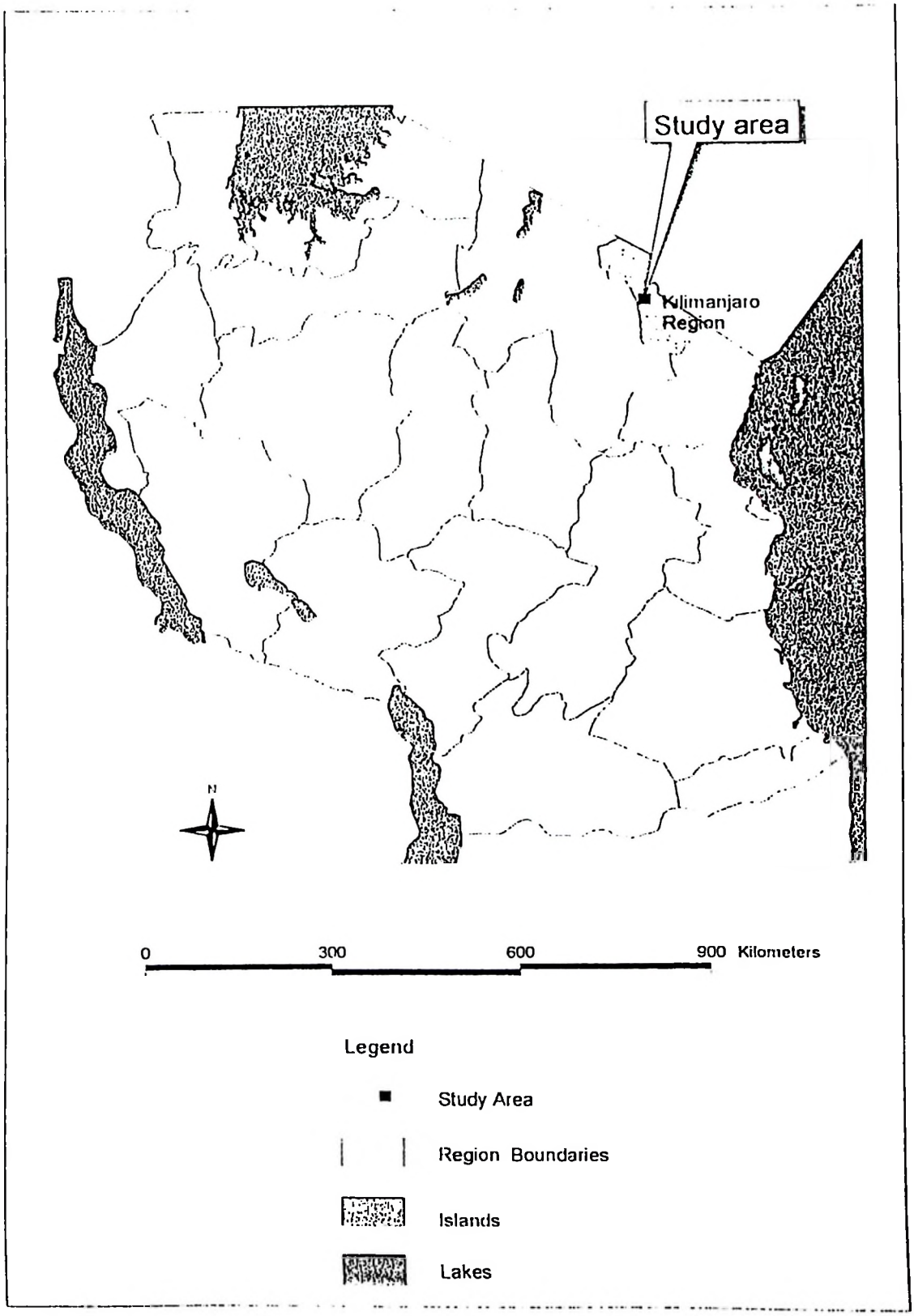

Figure 1. Map of Tanzania showing Kilimanjaro Region and location of TPC Sugarcane Estate.

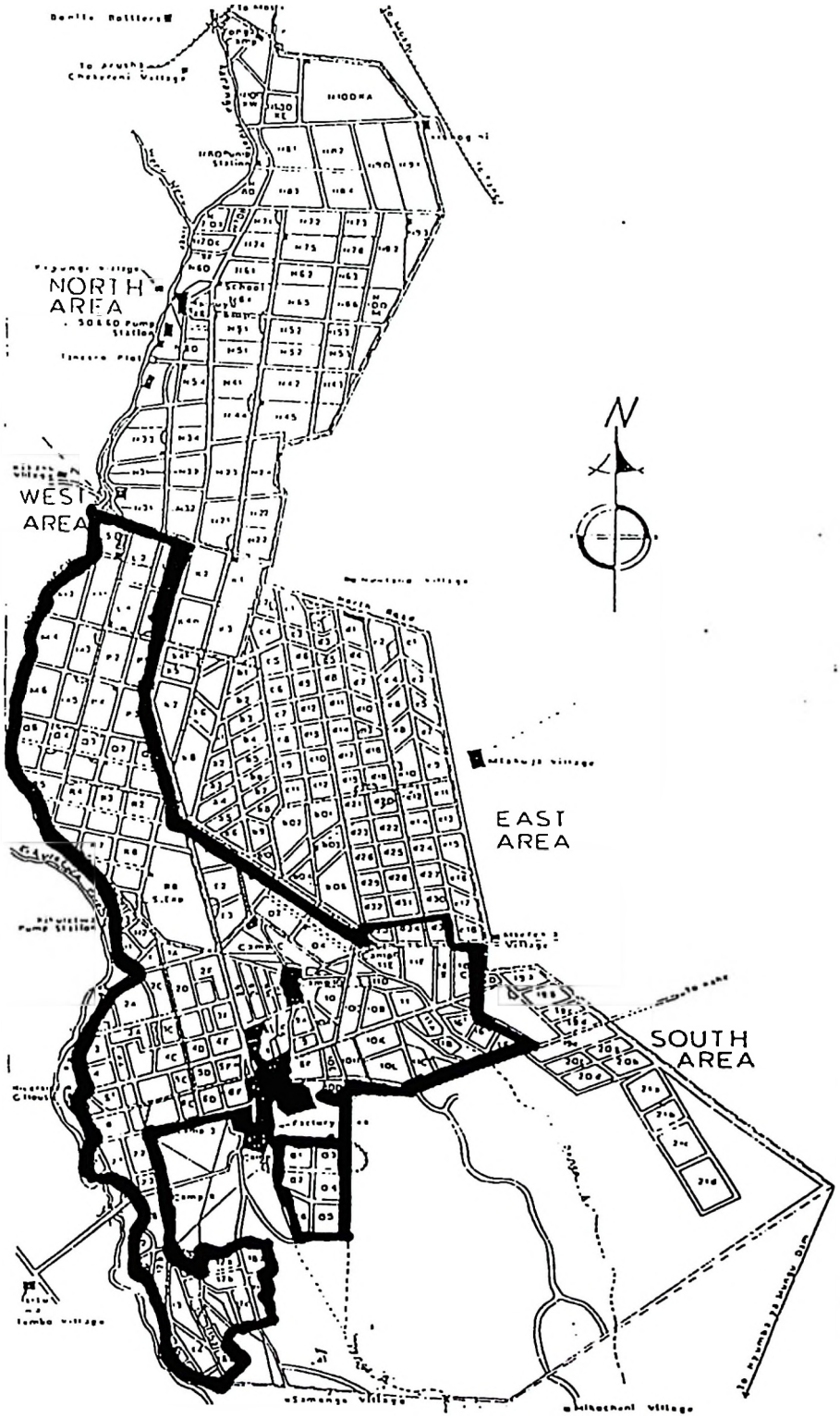

Figure 2. The map of TPC estate showing estate boundaries, bordering villages and white grubs infested fields.

The major commercial crop is sugarcane which is grown under irrigation all year round. This crop is produced solely by the TPC estate. Food crops grown by residents of Arusha Chini around the estate is maize which is grown as a sole crop and sometimes intercropped with beans or groundnuts. These crops are planted at the onset of the long rains, normally in mid March.

3.1.3 White grubs infested fields

From 30 to 50% of the area under sugarcane cultivation is heavily infested with white grubs. The distribution of this pest seems to be governed by soil type. The pest is found mainly in the western and southern parts of the estate, with some occurrences in the eastern part, where the soil textures are relatively light. The pest is usually not found in the northern part where the soils are heavy (clay).

3.2 Soil sampling and analysis

The soil samples used in this study were collected from the plough layer (0-30 cm) from the four selected fields which are under surface irrigation in the south and east area of the estate. These fields are known to be heavily infested by white grubs, and they have high incidences of *Cordyceps*. All the fields were planted with sugarcane variety B53-313 except for field 10D, which had a variety EA 70-97. The first field was 10D which had an area of 12.25 ha, in its second ratoon, Field G3, with an area of 21.25 ha in its fourth ratoon. Field 9 had an area of 17 ha, was in its fifth ratoon

and Field F1S was located in east area with an area of 30 ha and was in the eighth ratoon.

Soils were sampled both before and after fumigation with EDB. Additional samples were collected from those fields, which are usually not fumigated with EDB but they are known to harbour *Cordyceps* (field Q5s). From every field five sampling sites (four from each corner, and one at the middle) were sampled and the soil samples were mixed to obtain a compost sample. The samples were air-dried, ground and sieved through a 2 mm sieve. These soil samples were then used for routine analysis of selected physical and chemical properties.

Particle size analysis was done by the Bouyoucos hydrometer method as outlined by Day (1965). The pH of the soil was measured potentiometrically using the glass electrode in a 1:2.5 (w/v) soil:water suspension (MacLean, 1982). The soil organic carbon content was determined by the wet combustion method according to Walkley and Black as outlined by Nelson and Sommers (1982), whereas total N content of the soils was by the micro-Kjedahl method (Bremner and Mulvaney, 1982).

The electrical conductivity of the soils was measured using the electrical conductivity meter method as described by Van Reeuwijk (1987). The cation exchange capacity was done by sodium acetate method as described by Hesse (1971). Some physical and chemical properties of the experimental soils are presented in Table 1.

Table 1. Some properties of the experimental soils

Property	Field				
	G3	9	F1S	10D	Q_{ss}
pH	7.7	8.1	8.1	8.0	8.1
Organic carbon(%)	2.2	2.2	1.6	2.3	3.3
Total N(%)	0.11	0.10	0.07	0.13	0.01
CEC (me/100g soil)	63.2	62.8	54.8	65.2	58.8
ECe (1:5) dS /m	0.17	0.41	0.23	0.21	0.23
% Sand	79	84	74	76	70
% Silt	16	13	21	17	26
% Clay	5	3	5	7	4
Textural Class	LS	LS	SL	LS	SL

Textures:

SL = Sandy Loam

LS = Loamy Sand

The pH of the soils varied from 7.7 to 8.1. The organic carbon ranged from 1.64 to 3.29, which is rated as medium. Total N, ranged from 0.1 to 0.13, which was regarded as medium. The cation exchange capacity was high and the electrical conductivity level was that of normal soils. The loamy textural class, confirms that the soils in these fields were light, which should impart favourable physical conditions for sugarcane cultivation.

3.3 Determination of white grub population

The population of white grubs naturally occurring in the soil was determined using the pit sampling method as outlined by Chandarasacharan (1981) and modified by Katundu (1999). A rough plan of the field was made and it was divided into five sampling sites, one at each of the four corners and at the centre. Ten samples were taken from each marked site making a total of fifty samples per field.

Fields were sampled for all the stages of *C. melolonthoides* by digging pits of 1 m^2 and 0.5 m deep, giving a 0.5 m^3 as sampling unit. A pit included a sugar cane stool and portion of the inter-row space. The soil was removed and spread out outside the pit, where *C. melolonthoides* at different stages were hand-sorted from the soil and counted. These counts were used to calculate populations on per hectare basis. The pits were covered with the soil and flag marked for identification so as not to resample the same spot during subsequent counting.

3.4 Estimation of the populations of *Cordyceps* naturally occurring in soils using field transecting

In each of the selected fields, where white grubs occur, the population of the *C. barnesii* were estimated using the field strip transecting method described by Dent, (1997). Three locations were marked out, one at one corner of each field, the second at the centre of the field, and the third at the diagonally opposite corner. At each location, five points were designated in the cane inter-row spaces, each point separated from the other by five cane rows. At each point a person was placed who scouted a 50 m distance along the inter-row space, counting all emerged *Cordyceps* clavae. In this way, a total of 15 observations (counts) were made covering the designated three locations to represent the entire field. These counts were used to calculate the population of emerged *Cordyceps* per hectare. Such populations were obtained for all the five fields surveyed. The data was subjected to analysis of variance using the completely randomised design.

3.5 Source of white grub species to experiment for: Stress rearing

Third instar larvae of the red cane beetle (*Cochliotis melolonthoides* Gerst) used in this study were collected from grub infested fields. Since the collected larvae could be have been infected with *Cordyceps* without showing disease symptoms, all the healthy grub collections were first subjected to stress rearing to be sure that were free from *Cordyceps* and other pathogens infection.

Batches of 500 to 1000 third instar white grub larvae were randomly collected. The grubs were examined immediately after collection and all injured and/or showing disease symptoms were discarded. The healthy ones were reared under stress conditions (crowding and lack of food) in wooden trays without soil, but with abundant supply of moisture to prevent desiccation. The stress caused by removing the insect from their natural environment (soil) cause symptoms of pathogen infection to be more readily expressed. Signs of presence of *Cordyceps* infection in the grubs can be observed even before emergence of the *Cordyceps*. These signs may include abnormal behaviour such as poor co-ordination, jerky movements, excessive grooming and loss of orientation (Lomer, 1996). These were observed three to four days after imposing the stress. Those grubs with *Cordyceps* died within the first week with cadavers turgid, hard and mummified. External symptoms for larvae that were infected by bacteria include change in colour from normal white into darken to brownish black, often the larvae went flaccid without being liquefied. Those larvae infected by nematodes their body turned soft with the colour turning red (Lomer, 1996). The stress was maintained till the third week after which all the grubs which survived were deemed to be free from *Cordyceps* infection. These were resuscitated by placing in moist sand and by provision of food (maize plant roots). These were then used for further experimentation (section 3.9). After death the grubs became pink or yellowish to brown in colour, hard and mummified. Dead grubs (cadavers) were used in an experiment (section 3.9) to evaluate, in the laboratory, the effects of EDB on *Cordyceps* growth and development.

3.6 Effect of EDB on white grubs *in situ*

In each selected field with young sugarcane, a block of 240 m^2 was marked and divided into 12 plots of 20 m^2 each. Thus a total of six replicates of EDB treated and control plots were laid out randomly. EDB 45% was uniformly applied in treated plots using hand injector guns calibrated to give out the fumigant to the equivalent of 165 l/ha as currently applied by the estate management. Data on white grubs populations were taken before and at intervals of three, five and seven weeks after EDB application.

A commercial formulation of the EDB in kerosene was used. The trade name of fumigant is *Edabrom*. It contains 45% (w/w) active ingredient. With specific gravity of 2.180 at 20 °C, it is pale yellow in colour, flammable and stays in the liquid state below 40 °C (Appendix 6). This is the soil fumigant used routinely in the control of nematodes and wireworms in field crops in Israel (Israel Bromine Compounds, 1973). At TPC, this product is used to control the white grubs.

3.7 The effects of EDB on white grubs: laboratory studies

For these studies apparently healthy *C. melolonthoides*, which had survived the stress rearing (3.5) were resuscitated. Batches of ten grubs in four replicates were set up. The grubs were singly buried in pure moist sand which had been mixed with EDB as described in section 3.8. The rates of EDB used were equivalent of 0, 50, 100, or 200

litres per hectare. The equivalent rate of 150 l/ha could not be determined, due to shortage of healthy grubs post stress rearing. These grubs were supplied with live roots from maize seedlings, or germinating sugarcane vegetative material. These were checked before treatment, at the third, fifth, and seventh weeks after EDB treatment. Death of grubs was recorded and expressed as percentages. Data were analysed statistically, using the randomised complete block design.

3.8 The contribution of *Cordyceps* as biocontrol agents of white grubs

The role of *Cordyceps* as a natural mortality factor of white grubs was estimated using the trenching method outlined by Smith (1997). In each field, three diagonally spaced positions were marked. The first position was at one corner, the second in the centre of the field and the third at the diagonally opposite corner, as described in section 3.4. At each position a trench 20 m long and 30 cm deep was dug along the cane inter-row space. The soil so exposed was physically examined and all third instar grubs were counted. *Cordyceps*-infected larvae were determined. The number of *Cordyceps* infected grubs were determined and expressed as a percentage of all the third instar larvae (Dent, 1997). This gave an estimate of the level of natural control of white grubs by *Cordyceps* fungus in each of the four fields. This data was analysed statistically using the randomised complete block design.

3.9 Effects of EDB on *Cordyceps in situ*

Using the same field plots as described in section 3.6, *Cordyceps* populations were estimated using the field transecting method (section 3.4), before and at intervals of three, five and seven weeks after EDB application, with the objective of evaluating the effects of EDB on *Cordyceps* numbers in the course of time. Data were analysed for variance using the randomised complete block design.

3.10 Effect of EDB on *Cordyceps*: laboratory studies

For these studies white grubs which had died during stress rearing, with confirmed signs of *Cordyceps* infection, were used. Batches of ten cadavers were buried in, into moist pure sand contained in cups which had just been thoroughly mixed with EDB at the tested rates equivalent to 0, 50, 100, 150, or 200 l EDB /ha in four replicates prior to introducing the dead white grubs. Thus, the *Cordyceps* infected cadavers of grubs were exposed to these varying rates of EDB to evaluate the effect of the EDB on emergence of *Cordyceps* from the grub cadavers, as well as on the subsequent growth and development of the *Cordyceps*. The data recorded included the proportion of cadavers (out of the ten) from which *Cordyceps* emerged, (*Cordyceps* emerge from the head of the cadaver, at the junction of the frontal and coronal structure), the length as well as branching of the *Cordyceps* clavae. The data were analysed for variance using randomised complete block design.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Soils

The properties of the soils used in the present studies have already been presented (Table 1) and described in section 3.2.

4.2 White grubs

4.2.1 Occurrence of white grubs in TPC fields

The populations of white grubs in selected experimental fields are presented in Table 2. The populations differed ($p < 0.05$) between fields. Fields F1S and G3 had the highest white grub population densities. This was above the estate designated economic threshold level of 50 000 grubs per ha. The grub populations were found to be directly proportional to the number of sugarcane ratoons.

The difference in the occurrence of white grubs between the fields may be explained, in terms of spatial dynamics. These fields differed in their locations, sizes, crop stages, cane categories, cane varieties, soil types and time at which management

Table 2. Occurrence of white grubs in four selected experimental sugarcane fields

Field	Cane category (number of ratoons)	White grub populations/ 1 x 1 x 0.5 m^3 pit ± sd	Equivalent population per ha
G3	4	8.4 a ± 3.1	84 000
10D	3	4.9 ab ± 3.5	49 000
9	2	2.5 b ± 2.3	25 000
F1S	5	9.3 a ± 5.3	93 000
P – value		< 0.001	

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

options were administered. These variations perhaps provided, at times, different external stimuli which may have affected the populations of white grubs. Also by virtue of their locations, the different fields are different in soil types (Table 1), receiving different inputs and management options (such as irrigation and cultivation, fertilisers, herbicides, insecticides, fumigants etc.) at different periods of the year. Hence, almost each field has different conditions providing different external stimuli which would result in differences in behaviour of the grubs at any one time. This could be the reason for the differences in the occurrence and distribution of white grubs in the experimental fields.

Wyatt (1997) observed that single sensory inputs, such as chemical stimulus, had significant effects at each stage of insect development, and this may influence their behaviour from communication to host plant selection. Also different field operations (cultivation, harvesting, mechanical loading and transportation of sugarcane) which normally take some days to be accomplished in a particular field or location, and at different times of the year, might have forced the larvae to descend further into deeper soil levels, or adult beetles to migrate and thus change the egg laying locations.

Though grubs are phytophagous insects, they feed on organic matter during their first and second instar stages and later on they feed on plant roots. Different fields have at times different cane categories in terms of the number of ratoons (the number of times the crop has been allowed to regrow after harvest). Older ratoons normally have a higher concentration of root mass per unit volume of soil, which directly become easily available food for white grubs. More grubs are, therefore, found in fields with old ratoons, which provide ample of food for the grubs (Figure 3).

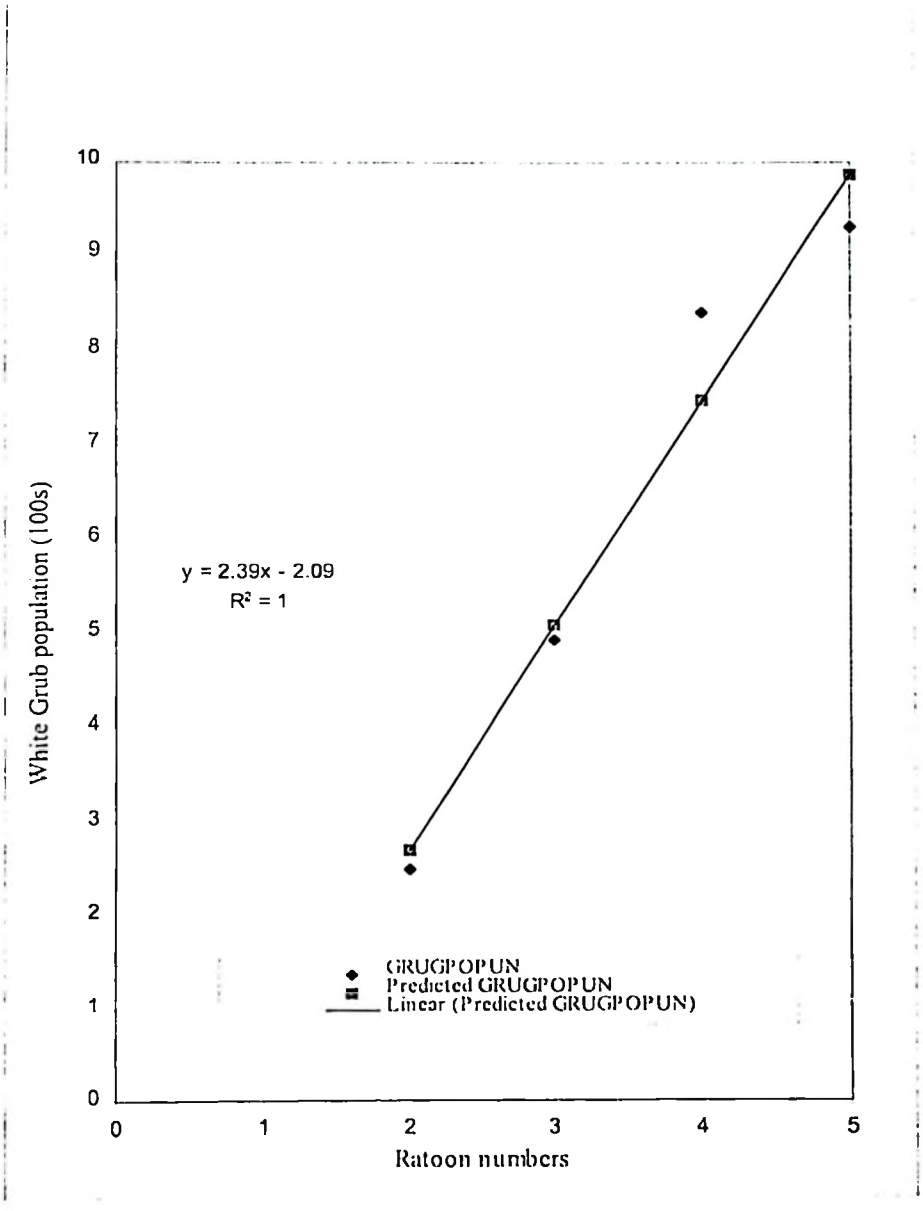

Figure 3. The relationship between white grub populations and number of cane ratoons.

4.2.2 Effect of EDB on white grubs in the field

4.2.2.1 Overall trends in four fields

The overall effect of EDB on white grubs in experimental fields is presented in Table 3. The general trend was that EDB reduced the white grub to population densities in treated field plots significantly ($p < 0.05$). Fields F1S and 10D had from their original white grub populations significantly reduced just three weeks after EDB had been applied, whereas field G3 had its population density reduced seven weeks after EDB application. The reduction in population density in field 9 was not significant.

The lack of significance of EDB in reducing the white grub population densities in field 9 can be explained partly by low initial density of white grub population and difficulty by the sampling technique in obtaining accurate estimates of populations. Also the soil physical properties especially the soil texture, could have attributed to the low EDB effectiveness. The field had the highest content of sand fraction (Table 1), which is known for its inverse relationship with the efficacy of the soil fumigants (Maloy, 1993). More of the fumigant was probably leached out of the root zone which is , characterised by high white grubs activities.

The significant reduction of white grub population densities by EDB in field F1S and 10D, could be explained by the fact that the fields had low content of the sand fraction and low organic carbon (which usually reflects low organic matter), which

Table 3. The effect of EDB on white grub population in four selected sugarcane experimental fields before and after EDB application

Field	G3	10D	9	F1S
Before EDB treatment	8.4 a ± 3.1	4.9 a ± 3.5	2.5 a ± 2.3	9.3 a ± 5.3
3 weeks after EDB treatment	4.1 a ± 7.8	1.6 b ± 2.2	1.8 a ± 2.2	0.3 b ± 0.5
7 weeks after EDB treatment	2.5 b ± 2.5	2.0 b ± 2.0	1.33 a ± 1.0	0.9 b ± 1.2
P-value	0.0368	0.009	0.4044	< 0.001

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

might have lead to little retention or adsorption of the fumigant. This might have made more of the fumigant to be available in the soil water and air interface. This free movement of EDB fumes, coupled with the initial high counts of grubs, increased the frequency of contact between white grubs and the fumigant, making the overall fumigation process more effective and resulting in high grub mortality.

4.2.2.2 Field by field trends of the efficacy of EDB

The effects of EDB on white grubs in each of the different fields are presented in Tables 4 through 8. From field G3, three weeks after EDB application, there was a significant difference($p < 0.05$) in grub population density, between treated and

Table 4 The effect of EDB on white grubs in field G3 before and after application

Treatment	Before application	3 weeks after application	7 weeks after application
Control	8.4 a ± 3.1	7.0 a ± 10.6	3.0 a ± 2.9
EDB 165 l/ha	8.4 a ± 3.1	1.7 b ± 2.2	2.0 a ± 2.2
p-value	1	0.001	0.554

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

control plots. The population density changed from 7.0 to 1.7 grubs per $0.5m^3$

The observed reduction of white grubs population densities at the third week after EDB application from field G3 may be due to the inherent properties of EDB, which include its low solubility, high volatility and its narcosis or narcotic mode of action. During EDB application soils were relatively "dry," giving large air passages for free movement of the fumigant and making it easier to come in contact with white grub larvae in the soil.

From field 9, the difference in white grub population between treated and untreated plots was observed to be statistically significant on the 7th week after EDB application (Table 5). In field 10D there was white grub population density reduction, but compared to the control plots, the difference was not statistically significant (Table 6). This may be due to soil physical properties (Table 1) and low

Table 5. The effect of EDB on white grubs in field No 9 before and after application

Treatment	Before application	3 weeks after application	7 weeks after application
Control	2.5 a ± 2.3	1.8 a ± 2.7	2.0 a ± 1.0
EDB 165 l/ha	2.5 a ± 2.3	1.8 a ± 1.9	0.7 b ± 0.5
p-value	1	1	0.124

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

Table 6. The effect of EDB on white grubs in field 10D before and after application

Treatment	Before application	3 weeks after application	7 weeks after application
Control	4.9 a ± 3.5	1.8 a ± 2.7	2.3 a ± 2.3
EDB 165 l/ha	4.9 a ± 3.5	1.3 a ± 1.2	1.8 a ± 1.9
p-value	1	0.101	0.693

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

population density of white grubs which was very difficult to estimate using the current technique. A similar trend was observed in field F1S (Table 7). The relationship between EDB efficiency (% grub kill) and percentage sand fraction is shown in Table 8 with regression square of 84%, in which efficacy of EDB against white grubs decreased as the amount of sand fraction increased in soils.

Table 7. The effect of EDB on white grubs in field F1S before and after application

Treatment	Before application	3 weeks after application	7 weeks after application
Control	9.0 a ± 5.5	0.33 a ± 0.5	1.5 a ± 1.5
EDB 165 l/ha	9.0 a ± 5.5	0.33 a ± 0.5	0.33 b ± 0.5
p-value	1	1	0.034

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

Table 8. Relationships between efficacy of EDB (% killing) on white grubs and some physico-chemical properties in soils of TPC estate

Regression equation	R^2	Probability value
% Grub kill = -8.1 + 1.2 * pH	0.25	0.76
% Grub kill = -2.59 + 1.96 * O C	0.43	0.35
% Grub kill = -2.58 + 12.25 Total N	0.11	0.60
% Grub kill = -13.86 + 0.2 * sand	0.84	0.09
% Grub kill = 5.89 + -0.26 * silt	0.85	0.08
% Grub kill = 3.15 + -0.32 * clay	0.32.	0.43

The lack of significant difference of EDB in reducing population densities of white grubs in this field can be explained both physical and chemical properties of the soil, especially the clay fraction and organic carbon content respectively (Table 8). High silt content might have reduced the efficacy of fumigant in soils and high organic carbon content which reflects high soil organic matter, retained through adsorption process, more of the applied EDB, thus making it little available for effective fumigation.

4.2.3 Effect of EDB on white grubs: Laboratory results

The laboratory results on the effects of EDB on the third instar white grub larvae are presented in Tables 9 and 10. More larvae were killed where EDB was applied at higher doses, equivalent to 200 l/ha (90). The untreated registered the lowest killing percentage (45%) with death trends being proportional to the sliding scale of EDB concentration. Higher doses were more lethal because of the increased concentration per unit volume of soil, which on turn increased frequency and contact to exposed larvae.

However, the killing percentages increased with time after EDB treatment. (Table 10). This suggests that three weeks is the minimum length of time required for effective control of white grubs fumigation using EDB. There after the efficacy was reduced probably because of either the evaporation of the EDB, or due to dilution

Table 9. Death (%) of third instars of white grubs treated with various rate of EDB in the laboratory

Treatment EDB l/ ha	Mean kill ± sd	Killed out of 40
0 (Control)	45.0 b ± 5.8	18
50	82.5 a ± 22.2	33
100	70	28*
150	n.d	-
200	90.0 a ± 8.2	36

n.d = not determined.

* = Only single replicate was determined and was not put into statistics.

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

effect caused by water that were added to prevent the larvae from desiccation and supplying moisture to the maize seedlings that were used to provided to feed the experimental grubs.

Table 11. Occurrence of *Cordyceps* in four selected experimental sugarcane fields at the TPC estate

Field	*Cordyceps* populations/ 1.46 x 0.3 x 20 m^3 trench	Equivalent number Per ha
G3	8.7 ab ± 4.6	2979
10D	11.7 a ± 8.1	4007
9	2.3 b ± 3.6	787
F1S	1.7 b ± 1.0	582
P-value	0.0055	

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test

These factors include host density or populations, inoculum pressure and soil moisture (Robert *et al.*, 1988). Fields 10D and G3 had relatively higher incidences of white grubs, which are the hosts of *Cordyceps* Field F1S had the lowest *Cordyceps* but high grubs count. This may be due to the following reasons; The relative high pH in soils of F1S field may have contributed to the lower *Cordyceps* populations and the relative lower pH values of fields 10D and G3, closer to the pH of 7 (Griff, 1940), may have favoured maximum development of *Cordyceps*, increases the vulnerability of grubs to fumigants making the population not stable. Since *Cordyceps* grows specifically on white grubs, any disruption of white grubs population therefore, should have a greater effect on *Cordyceps* fungal as specific pathogen. The linear relationship between *Cordyceps* populations and pH of the soils is presented in Appendix 7.

4.4 The contribution of *Cordyceps* in the biocontrol of white grubs

Table 12 presents the proportions of different stages of white grubs in the four experimental fields during the field surveys in November 1999. On the average about 69 (range of 54-94) of all third instar larvae were found to be parasitised by *Cordyceps* in the surveyed fields Table 13.

Per cent parasitism of third instar larvae determined by collection of host samples alone (stress rearing) in the laboratory are presented in Table 14. with a general mean of 8.6% (range of 0.25-27%) of collected third instar larvae were found to be infected by *Cordyceps* after incubation. Similarly, an infection rate of 3.5% by *Cordyceps* was observed by Minja *et al.*,(1993), while Katundu (1999) observed a range of 10 – 25%.

Third instar larvae occupied one sixth of all the other stages in the soil and it is the most destructive stage. Per cent parasitism of 3rd instar white grubs by *Cordyceps* determined by strip transects in the field are presented in Table 13. It follows then from the field surveys and laboratory collections that *Cordyceps* as biological agents at the TPC estate, contributes significantly in controlling white grubs. The stress rearing data in the laboratory (Table 14), suggest that percentage infection of grubs obtained through transect method could be even higher in the field. Also death of white grubs in the filed due to nematodes and bacteria seems to be equally significance and gives promising clues for further study along that line.

Table 12. Proportions of different stages of *Cochliotis melolonthoides* in four experimental sugarcane fields at the TPC estate in November 1999

Field	Eggs	1st instar	2nd instar	3rd instar	Pupa	Adults	3rd: others
G3	0	8	9	6	0	0	1:3
10D	0	6	17	4	0	0	1:6
9	0	3	3	5	0	0	1:1
F1S	0	22	22	2	0	0	1:22
Totals	0	39	51	17	0	0	1:5
Mean	0	10	13	4	0	0	1:6

Table 13. *Cordyceps* infection on 3rd instar larvae of white grubs in four experimental sugarcane fields at the TPC estate

Field	Healthy 3rd instars in 0.3 x1.46 x 20 m^3 trench	*Cordyceps* infected 3rd instars in 0.3 x1.46 x 20 m^3trench	% *Cordyceps* infected 3rd instars in 0.3 x1.46 x 20 m^3trench
G3	22	26	54
10D	2	35	94
9	5	7	58
F1S	3	5	62
Totals	32	73	69
Mean	8	18.2	69.2

Table 14. Stress rearing of 3rd instar white grubs

Batch number	Total collection	Dead due to *Cordyceps* (% of total collection)	Dead due to nematodes	Dead due to Bacteria	Dead due to other causes
1	228	29 (12.7%)	3	155	101
2	228	38 (16.7%)	1	188	62
3	2258	112 (5.%)	8	247	1390
4	879	239 (27.2%)	0	182	458
5	928	141(15.2%)	1	431	355
6	2015	5 (0.25%)	0	847	1154
Totals	6536	564	13	2050	3520
Percentage	-	8.6	0.2	31.3	53.8

4.4.1 Effect of EDB on *Cordyceps* in the field

4.4.1.1 Overall trends in the four experimental fields

The overall effects of EDB on *Cordyceps barnesii* in the four experimental fields are presented in Table 15. There were significant ($p < 0.05$) reductions in *Cordyceps* populations in fields G3 and 10D, but not in fields 9 and F1S. The population was reduced from 8.7 to 1.3 and 11.7 to 3.7 *Cordyceps* per (20 x 1.46 x 0.3m^3) trench for fields G3 and 10D respectively. However, population increased from 2.3 to 5.0 *Cordyceps* per (20 x 1.46 x 0.3 m^3) trench in field 9 and it remained fairly constant at 1.7 *Cordyceps* per (20 x 1.46 x 0.3 m^3) trench in field F1S, three weeks after EDB application.

Table 15. The effect of EDB on *Cordyceps* in the four selected experimental fields before and after EDB application

Field	G3	10D	9	F1S
Before EDB treatment	8.7a ± 4.6	11.7a ± 8.1	2.3 a ± 3.6	1.7a ± 1.0
3 weeks after EDB treatment	1.3 b ± 1.2	3.7 b ± 2.7	5.0 a ± 8.4	1.7 a ± 1.2
7 weeks after EDB application	1.5 b ± 1.4	1.5 b ± 1.4	0.0 a ± 0	2.2 a ± 2.6
P-value	< 0.001	0.0072	0.2922	0.6163

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

From this field the populations of *Cordyceps* started to build up at the seventh week after EDB application.

The significant ($p < 0.05$) reduction in *Cordyceps* population in field G3 and 10D can be explained in terms of the biocidal effect exerted by EDB as a fumigant and the interaction between soil physico-chemical properties and the fumigant that influenced the availability of white grubs, the specific hosts of *Cordyceps*.

EDB as a weak acid is almost invariably more effective at relatively lower pH than high (Cochrane, 1958). Fields G3 and 10D had relatively lower pH values [though not on the acid side of the pH scale (Table 1)] than from fields 9 and F1S, and that the pH values were very close to the pKa value, where the effect of pH is most noticeable (Cochrane, 1958). It is known that only the undissociated molecule is toxic as it enters the cells of the receptor organ more rapidly than its ions (Cochrane, 1958).

The build up of *Cordyceps* populations three weeks after EDB application from field F1S can be explained by structurally non-specific nature of EDB as narcotic poison for fungi, which is a freely reversible (Cochrane, 1958). The biological effectiveness of EDB therefore, was a function of proportional concentration at the site of action, with toxicity depending on heterogeneous soil phases (soil air, soil water) and fungal protoplasm.

Papavizas and Lewis (1979), pointed out that insecticides and nematicides may indirectly affect soil borne pathogens by increasing or destroying the predisposing factors such as non-target antagonistic microorganisms. In a critical review on the effect of biocidal treatment on soils micro flora, Lester (1979), pointed out an initial decrease in population of fungi followed by an increase to a level exceeding that in the untreated soil.

The lack of a significant effect of EDB in reducing *Cordyceps* populations in fields 9 and F1S, can be explained in terms of chemical properties of soil especially higher soil pH and per cent sand fraction (Table 1), which may have changed EDB's fungicidal effect and its availability, respectively, in those fields.

4.4.2 Effect of EDB on *Cordyceps*: Laboratory results

Laboratory results of the effects of EDB on *Cordyceps* development (emergence, growth and branching) are presented in Table 16. Although there was a statistical significant ($p < 0.05$) difference in the emergence of *Cordyceps* clavae only between control (75%) and the highest rate (200 l/ha) of EDB treatment (20%), generally the results showed that emergence of *Cordyceps* decreased with increasing EDB rates.

There were significant but inconsistent differences in growth of *Cordyceps* clavae from cadavers buried in soils treated with varying rates of EDB. The maximum growth rate (7.2) obtained at the rate equivalent of 50 l/ha could not be explained. However, the high rate of 200 l/ha which registered the lowest growth of 0.6 cm could be due to the inhibition effect of EDB on the fungal growth.

There were no significant ($p > 0.05$) differences in branching of *Cordyceps* clavae in soils treated with varying rates of EDB, but the general trend was the decrease of branching with increasing rates of EDB.

The demonstrated significant difference in emergence of *Cordyceps* clavae from the *Cordyceps* infested cadavers, may be explained by the dimorphic property of *Cordyceps* as regards cellular morphogenesis, in which a reversible transformation from a mycelial to a non mycilial growth type occurs.

Table 16. *Cordyceps* clavae development after treating with varying equivalent rates of EDB in the laboratory

EDB equivalent rate (l/ ha)	Mean emergence ± sd	Mean growth (cm) ± sd	Mean branching ± sd
0 (Control)	75.0 a ± 12.9	3.0 bc ± 0.8	45.0 a ± 34.2
50	47.0 ab ± 5.0	7.2 a ± 1.5	15.0a ± 12.9
100	32.5 ab ± 18.9	2.2 bc ± 0.7	12.5 a ± 12.5
150	45.0 ab ± 10.0	3.9 b ± 2.3	12.5 a ± 5.0
200	20.2 b ± 18.3	0.6 c ± 0.6	13.7 a ± 14.9

Means within the same column followed by the same letter(s) are not significantly (p = 0.05) different according to Tukey's multiple comparisons test.

It is known in principle that stimuli are known to affect dimorphism in pathogenic fungi, and act through their effect on the sulfhydryl content of the cells (Cochrane, 1958). So, the EDB applied at varying rates could have been accumulated in different concentrations around the emergence points, affecting differently the emergence of *Cordyceps* clavae from the infected dead larvae. Inhibitory effects of growth media in fungi act only on cells that are in contact with that media (Cochrane, 1958). However, the growing part of the *Cordyceps* clava is an extended portion of the mycelial tip. So, the EDB, which was in contact with the cadaver, might probably not have affected directly the growth rate of the growing tips, which were largely out of direct contact with the EDB. This may have resulted in some uniformity or similarity of growth across EDB treatments.

The lack of significant differences in branching and inconsistency in the growth pattern showed by emerged clavae from white grub cadavers with varying rates of EDB can be explained by uniformity in the decline of nutrient supply. After killing the grubs through infection, *Cordyceps* start to live saprophytically (Robert *et al.*, 1988), with the cadaver being the only source of nutrients. All the cadavers were of the third instar larvae with more or less similar size, age and mass. So it was possible that the growing *Cordyceps*, would have had the same/equal rates of nutrient consumption, causing no differences in their growth rates. Therefore EDB had no effect on the branching.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY AND CONCLUSIONS

White grubs and *Cordyceps* occur at different population levels and proportions, with the populations of both the host and pathogen being higher in sugarcane fields with old ratoons. EDB when applied in soils reduced populations of both white grubs and *Cordyceps* in different proportions. More populations of both white grubs and *Cordyceps* were reduced in the first three weeks after EDB application, with reduction being more in soils with low carbon content and low percentage of clay fraction than in sandy soils.

In the laboratory, EDB reduced the populations of white grubs at higher rates with reduction being consistent to the sliding scale of EDB concentration. EDB affected significantly the emergence and slightly the growth but didn't affect the branching of growing *Cordyceps* clavae for all the equivalent rates applied.

From the results of the present study, it is tentatively concluded that:

1. Both white grubs and *Cordyceps* occur in different population levels and proportions in different sugarcane fields, because of the differences in the conditions that favour their occurrence.

2. When used at the recommended rates under favourable soil conditions, EDB reduced the population of both white grubs and *Cordyceps*.

3. Organic carbon content, sand and clay fractions of the soil seem to be dominant factors that affect the efficiency of EDB in controlling white grubs

4. Once they have emerged from cadavers, *Cordyceps* appear to have been affected to some extent by EDB applied in the soil.

5. At TPC estate, residual effect of EDB in the soil clearly is shown in the first three weeks after application.

6. Depending on time and method of sampling, *Cordyceps* have significant contribution in controlling white grubs in sugarcane fields.

5.2. RECOMMENDATIONS

From the findings of the present study, the following can be recommended;

(i) Since EDB reduces the populations of not only white grubs as target pest but also that of *Cordyceps*, emphasis need to be further assessment of the impact of this chemical on other non target organisms and natural enemies.

(ii) Further studies are required to develop other alternative control strategies which are more sustainable with less adverse impact to natural enemies and the environment.

CHAPTER SIX

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APPENDICES

Appendix 1. Partial list of insecticides used against white grubs at the TPC estate (1940s - 1990s).

Period	Insecticide	Application rate per ha	Target insect stage
1946- 1950s	BHC (2.5)	205 kg a.i	Larva
1950-1970	Aldrin (40 WP)	1.12 kg a.i	Larva
1970s	Trichloronate (2.5%) + phoxim (7.5%)	2.5 kg a.i	Larva
1980	Ethylene dibromide (EDB 45%)	125- 165 l	Larva
1993	Chlorpyifos (SusCon Blue 14G)	42 kg	Larva
1994	Isazofos (Miral 10G)	40 kg	Larva
1994	ethoprop (Mocap 10 G)	40 kg	Larva
1980s..	dichlovos (Nogos Ulvair 250)	40 kg	Adult
1990s	Cypermethrin (Ripcord)	2.5 l	Adult
1994	Profenofos + cypermethrin *high-Cis* (Fenom C)	2.5 l	Adult

Source: Katundu, 1999.

Appendix 2. Quantity and cost of Ethylene dibromide applied at the T P C estate 1984 - 1998 period.

Cropping season	Quantity (Litres)	Unit price (TShs)	Total cost (TShs)
1984 /85	18 900	600	11 340 000
1985 /86	156 400	600	93 840 000
1986 /87	142 200	600	85 320 000
1987 /88	120 000	700	84 000 000
1988 /89	149 700	700	104 790 000
1989/90	64 000	700	44 800 000
1990 /91	175 900	800	140 720 000
1991 /92	142 700	800	114 160 000
1992 /93	303 300	800	242 640 000
1993 /94	88 000	1 170	102 960 000
1994 /95	60 000	1 170	70 200 000
1995 /96	43 800	1 170	51 246 000
1996 /97	60 800	1 332	80 985 600
1997 /98	3 400	1 250	4 250 000
TOTAL	1 529 100	1 392	1 231 251 600

Source: TPC stores and purchasing office.

Appendix 3. Seasonal white grub infestation and sugarcane yield at the TPC estate (1979-1992)

Year	Cane yield (TCH)	Number of fields	Economic Threshold at	Ploughing up	Fumigation %
1979	95	45	75	20	48
1980	100	80	56	6	24
1981	110	117	44	7	17
1982	113	99	32	5	10
1983	110	110	30	4	13
1984	119	86	50	14	23
1985	112	92	60	18	32
1986	98.4	88	81	22	57
1987	80.4	107	61	4	34
1988	68.9	109	73	6	36
1989	52.5	81	78	21	32
1990	69.5	120	68	10	17
1991	60	114	73	19	19
1992	70	113	60	15	19

TCH = tons of cane per ha.

Economic threshold = white grub density of 50 00 grubs of all the stages per hectare.

Ploughing up = Fields with more than 100 000 white grubs per ha recommended for ploughing up.

Fumigation = Field with more than 50 000 white grubs per ha are fumigated with EDB

Source: J. M. Katundu 1999.

Appendix 4. EDB use and its efficiency in grubby fields at the TPC estate

		Grub count after rounds of fumigation			Quantity of EDB applied (litres)		Application rates (litres)	Variance
Field	Hectares	At harvest	1st round	2nd round	1st round	2nd round		
O3	8.00	104 245	99 463	-	800	-	100	0
O3	8.00	-	99 463	126, 721	-	800	100	0
R7	34.32	61 687	73 162	-	3600	-	100	+ 168.0
R7	34.32	-	73 162	113,330	-	360	100	+ 168.0
BO5	32.74	79 857	64 077	-	3300a	-	100	+ 26.0
I	12.54	36 342	77 946	-	1300	-	100	+ 45
F3	38.00	53 557	52 600	-	3900	-	100	+ 100.0
10E	8.22	82 727	40 646	-	900	-	100	+ 78.0
Q1N	25.07	154 934	13 867	-	4200	-	165	+ 63.45
M4	39.74	52 123	18 997	-	4000	-	100	+ 26.0
11D	13.15	201 757	31 081	-	2200	-	100	+ 885.0
10C	24.64	105 679	10 042	-	2600	-	100	+136.0
Total	-	932 909	654 506	-	-	-	-	+1695.45
Mean	-	93 290.8	54 542.08	-	-	-	-	+141.28

Source: TPC Limited. Agronomy Office.

Appendix 5. Mean annual Climatic data, of the TPC area

Year	Rainfall (mm)	Temperature. (c^0)	Humidity. (%)	Sun shine (hrs)	Evaporation. (mm)	Solar radiation.
1987	515.1	25.0	83.0	7.5	215.2	582.3
1988	572.4	25.3	83.0	7.0	23.0	570.1
1989	595.5	24.5	81.0	6.4	222.6	543.2
1990	1142.2	24.3	81.0	6.8	207.3	554.1
1991	322.5	23.1	79.0	7.3	271.2	573.7
1992	351.6	24.8	76.0	6.6	200.2	585.2
1993	271.9	24.6	77.0	7.0	189.1	584.8
1994	385.9	24.6	76.0	6.4	187.0	549.0
1995	671.8	24.8	78.0	6.9	187.0	581.0
1996	579	25.1	82.0	6.0	197.7	558.0

Source: TPC meteorological station.

Appendix 6. Some physical and chemical properties of EDB.

Chemical name	1, 2-dibromoethane
Chemical formula	$C_2H_4Br_2$
Molecular weight	187.8
Grade	Technical
Purity	99.5%
Specific gravity at 20 °C	2.180
Distillation range at 760 mm Hg	3 °C to include 131.7(min. 90 of material)
Freezing point	9.2-9.7 °C
Flash point T.O.C.	None
Colour	Colourless and clear
Odour	Sweetish smell
Vapour pressure at 20 °C, mm Hg	10
Vapour density (air = to 1)	6.5
Solubility	Slightly soluble in water (0.43) at 30 °C; Miscible with alcohol, ether and kerosene
Acidity (as HBr)	Less than 10 ppm. May increase with Prolonged storage
Stability	Stable in solutions with hydrocarbons °0 Solid formulations. Reacts with dilute Inorganic bases and active metals
Maximum allowable concentration by volume in air for an 8-hour working exposure	25 ppm

Source: Israel Bromo Compounds, 1973.

Appendix 7. The relationship between pH and *Cordyceps* in soils of the TPC estate.