INVESTIGATION OF THE FUNGAL COMMUNITY ON DISEASE ROOT AT SABAH TEA PLANTATION

NUR AZURA BERAHIM

DISERTASI YANG DIKEMUKAKAN UNTUK MEMENUHI SEBAHAGIAN DARIPADA SYARAT MEMPEROLEHI IJAZAH SARJANA MUDA SAINS DENGAN KEPUJIAN

PROGRAM SAINS SEKITARAN
SEKOLAH SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA SABAH

2005

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: INVESTIGATION OF THE FUNGAL COMMUNITY ON DISEASE ROOT AT SABAH TEA PLANTATION

Ijazah: SARJANA MUDA SAINS DENGAN KEPUJIAN (SAINS SEKITARAN)

SESI PENGAJIAN: 2002/2005

Saya NUR AZURA BERAHIM

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[/]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: LOT 7, JLN 4/3K, FASA 4, 43650 BANDAR BARU BANGI, SELANGOR.

Nama Penyelia

Tarikh: 24 Mac 2005

Tarikh:

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

ACKNOWLEDGMENT

I confess this work is produced by me except for summary and statement that have been clarified the references.

21 February 2005

NUR AZURA BERAHIM

HS 2002/3908

ACKNOWLEDGED BY

Signature

1. **SUPERVISOR**
   (DR. ANJA GASSNER) _____

2. **EXAMINER 1**
   (DR. BONAVENTURE VUN LEONG WAN) _____

3. **EXAMINER 2**
   (DR. MIROSLAV RADOJEVIC) _____

4. **DEAN**
   (PROF. MADYA DR. AMRAN AHMED) _____

APPRECIATION

I am especially grateful to the Almighty that finally I have completed this dissertation for fulfil the requirement of Bachelor of Science (Hon.). It is important to thank my beloved supervisor, Dr.Anja Gassner for her big contributions. I am grateful for her guidance, advice and big support. Without whom this dissertation has been impossible. Thanks also to Sabah Tea Plantation for offering a financial support to this research project.

A big thankful for my beloved parents, Hj. Berahim Ithnin and Hjh. Aini Yahya for all their full belief and supported to me. To my brothers, Nazrul and Nazer, my sisters, Nur Aziah and Nur Aisyah, thank for all your actuation and motivation.

My gratitude also goes to Prof. Madya Dr. Marcus Atong, Plant Technology Program lecturer for his advice and who provided significant comments and suggestions to this research. Not forgotten to our laboratory staffs at School of Science and Technology, UMS especially Miss Christina Kungin and Mr. Sanin Awang for their help in the manual preparation.

Appreciation is also due to Miss Nurul Hidayah, forestry student for her taught and assistance. To Miss Noor Afidah Md.Zulkepli, thank for your encouragement and accompany in a labwork. Last but not least I would like to thank my Environmental Program colleagues especially Niena, Yam, Fai, Jeremy, Zairul and the others for their encouragement and support for this dissertation.

Thank you.

NUR AZURA BERAHIM

ABSTRACT

The study was carried out at Sabah Tea Plantation which is located at 20 km from Ranau at Kampung Nalapak, Sabah. The study is to identify the causal agent of the root rot disease occurring at Sabah Tea. 14 root samples of infected tea trees, believed to have died of *Poria* disease were collected. Whereby, 7 root samples were taken from a field under conventional farming practice and 7 from a field under organic farming practice. Extraction of pathogens was carried out by surface sterilization and subsequent culturing on PDA. Out of the 14 root samples, funguses were successfully extracted from 9 samples, whereby 4 different types were found. Based on microscopic examination the causal agent of the disease attacked could not be confirmed. It was therefore concluded that the disease at Sabah Tea was not caused by *Poria Hypolateritia*. Instead it is suggested that it is a brown root rot, caused by the fungus *Fomes noxius*.

ABSTRAK

KAJIAN KOMUNITI KULAT PADA PENYAKIT AKAR DI LADANG TEH SABAH.

Kajian telah dijalankan di Ladang Teh Sabah yang terletak di Kampung Nalapak, 20 km dari Ranau. Kajian telah dijalankan untuk mengenalpasti agen penyebab serangan penyakit akar di ladang Teh Sabah. 14 sampel akar yang dipercayai telah dijangkiti oleh penyakit *Poria Hypolateritia* telah diambil. Sebanyak 7 sampel akar telah diambil daripada ladang konvensional dan 7 sampel lagi diambil daripada ladang organik. Kaedah yang digunakan untuk mengekstrak patogen adalah melalui kaedah pengkulturan steril akar dan subkultur akar di dalam media Potato Dextrose Agar(PDA). Hasil yang diperolehi dalam kajian ini ialah 9 sampel telah berjaya diekstrak dan empat jenis kumpulan kulat telah ditemui. Berdasarkan pemerhatian daripada mikroskop, agen penyebab serangan penyakit kulat tidak dapat disahkan. Secara keseluruhannya, telah dicadangkan bahawa serangan penyakit kulat di Ladang Teh Sabah adalah bukan disebabkan oleh *Poria Hypolateritia* sebaliknya disebabkan oleh kulat dari spesis *Fomes Noxius*.

TABLE OF CONTENT

LIST OF TABLE

LIST OF FIGURES

LIST OF SYMBOL AND ABBREVIATION

HCL	Hydrochloric acid
H_2O	Water
KOH	Calcium Hydroxide
°C	Degree Celsius
°F	Degree Fahrenheit
Km	Kilometer
Kmh-1	Kilometer per hour
M	Meter
cm	centimeter
mm	millimeter
µm	micrometer
ml	milliliter

CHAPTER 1

INTRODUCTION

1.1 Introduction

In Malaysia, there are two large tea plantations. The larger one is situated in Cameron Highland, Pahang Darul Makmur. Whereas the other tea plantations Sabah Tea is located close to Ranau in Sabah. Sabah Tea is the largest single commercial tea plantation in Sabah. Sabah tea is located at the foothills of Mount Kinabalu in Ranau, surrounded by the world's oldest rainforest which is rich and full of flora and fauna.

Optimal yield production at Sabah Tea is hampered by the presence of root diseases. Until now the plantation has not identified what kind of root rot disease is killing the tea trees. The causal agent of this disease is thought to be *Poria hypolateritia*, a soil born pathogen. Root rot has spread widely in the tea field of Sabah Tea. Even though this root rot disease is the most dangerous disease for the tea plantation, there is at present no sustainable control of the spread of the disease. Fumigation of infected areas with methyl bromide although very successful is not applicable with an organic farming system. The present management procedure, more environmental sound, consists of digging up the root of affected tea trees and removing the infected tea tree together with one or two rows of surrounding

apparently healthy trees. In addition Guatemala grass (*Tripsacum laxum*) is planted to reduce the *Poria* spreading as this grass is known to interrupt the life cycle of *Poria.* However, this practice needs labour energy and is time consuming and does not always prevents the spread of the disease and is not that successful in reducing the spreading of the disease.

The proposed project is to develop and implement an effective integrated pest management strategy (IPM) for the red root rot disease at Sabah Tea. To find the best and effective way for a good management strategy to control the root rot disease we have to understand the strains that are causing the root rot, the behavior and characteristic of the fungus.

1.2 Objectives

The overall objectives of the propose study is to identify the causal agent of the root rot disease occurring at Sabah Tea.

Meanwhile, to achieve this goal, following task will be carried out.

1. Extract fungus from infected root samples.
2. To confirm if the presence of *Poria Hypolateritia* on infected root samples.
3. To investigate the effect of management practices between conventional and organic farm on the fungi community of disease tea tree roots

CHAPTER 2

LITERATURE REVIEW

2.1 Plant disease epidemiology

The epidemiology of the plant disease is important to get a better understanding of disease problems. Plant disease epidemiology is the most effective approach to get a solution of disease problems. Epidemiology is the overall study of epidemics, the comprehensive analysis of the interaction between the three constituents of the disease triangle; the host, the pathogen and the environment (Jones, 1998). These disease triangles are related to each other in spreading disease problem and there is the primary requirement for the development of a disease. A causal agent and a favorable environment constitute two of the three requisite factors for the occurrence of infectious plant disease (Kenaga, 1974). Firstly, the pathogen must be present; secondly the host plant must be susceptible to the particular pathogen; thirdly the environment including soil and weather conditions must be favorable for infection to take place and for spread to occur (Martin, 1972).

Most of the plants are liable to be attacked by several and sometimes many different pathogens. Some pathogens can be recognized by the characteristic symptoms that they produce. Most of the plant pathogens belong to one of the three groups; fungi, bacteria and viruses and all of these groups have their main characteristics.

The fungi causing plant diseases can be grouped into four sub-groups. Fungi are separated into four classes on the basis of the morphology of their sexual structures and the sporulating organs formed after sexual reproduction (Strange, 1993). Besides that, these sub-groups are distinguished from one another by the microscopic characters of the mycelium (Martin, 1972). These are the Ascomycetes, Basidiomycetes, Phycomycetes and Zygomycetes.

2.2 Basidiomycetes

The fungi comprising the phylum Basidiomycota are commonly known as basidiomycetes. Basidiomycetes are a large and diverse group and include forms commonly known as mushrooms, puffballs, jelly fungi, bracket or shelf fungi and rust and smut fungi (Alexopoulos, et al., 1996). Basidiomycetes are characterized primarily by the fact that they produce their sexual spores, termed basidiospores, on the outside of a specialized, microscopic, spore- producing structure called the basidium (Alexopoulos, et al., 1996).

Meanwhile, the fungus that infected tea plantation is in basidiomycetes group (Alexopoulos, et al., 1996).The basidiomycetes are an important group of fungi including harmful as well as useful species. They also often produce two or more kinds of spores but one is always formed on a characteristic structure and known as a basidium. This group includes the smuts represented by loose smut of wheat and barley and the rusts, species of which can be found on most plants (Martin, 1972). Notorious among these diseases are stinking smut and black stem rust of wheat (Alexopoulos, et al., 1996). Many others attack a large variety of food and ornamental

plants. Besides that, several basidiomycetes are also significant in causing diseases of forest and shade trees (Alexopoulos, et al., 1996). Their causal relation between fungus and disease is relatively easy to prove as the characteristics spores, which they are usually produced in profusion, can be collected and used as inoculums for further plants. Developments of similar symptoms to those of the original diseased plant are strong evidences that collected fungus is causing the disease.

2.3 Soil Borne Pathogens

Soil borne pathogens can be defined as those which undertake the major part of their life cycle in the soil (Hillocks & Waller, 1997). Typically, they infect roots or stem bases; dispersal and survival stages are primarily confined to the soil. Besides that, soil borne pathogens may also produce air or water dispersed spores which result in dispersal over larger areas.

A system of classifying the soil borne fungal pathogens is required to get the best approach to control diseases. There are two ways of grouping the soil borne fungi. One is based on their mode of parasitism and divides them into specialized and non-specialized parasites. The other is based on ecological criteria and groups them into soil inhibitors or soil invaders (Hillocks & Waller, 1997). The soil-inhibiting fungus is characterized by its ability to survive as a saprophyte and this is a specialized parasites. The soil-invading or root-inhabiting fungus is s non-specialized parasites characterized by an expanding parasitic phase and a declining saprophytic phase after the host's death.

The soil-invaders exhibit a greater degree of parasitic specialization than the soil –inhibitors and therefore have a more restricted host range. They are divided into three groups according to their aetiology; one is the vascular wilt pathogens; e.g *Fusarium oxysporum*. These pathogens are confined to the vascular tissue after initial invasion (Hillocks & Waller, 1997). Second are ectotrophic root-infecting fungi. These ectotrophic root pathogens are able to grow extensively on the outside of the root and can penetrate the root cortex. Many of them are liberated from dependence on survival in the soil by their ability to produce rhizomorphs or specialized mycelial strands, which can grow from plant to plant along adjacent roots; e.g *Armillaria mellea* .(Hillocks & Waller, 1997). The third group is the obligate parasites which produce motile zoospores; e.g *Plasmodiophora brassicae*. This group is not well represented in the tropics and these pathogens are mostly confined to the Chytridiomycetes, Oomycetes and Plasmodiophorales (Hillocks & Waller, 1997).

2.4 Root Disease

Root diseases of woody species are caused by a group of fungi that exhibit some degree of pathogenic specialization and spread from plant to plant by rhizomorphs which can usually be found under the bark of trees in the advances stages of infection. These fungi mainly belong to the genera *Fomes*, and *Armillaria* among the basidiomycetes but similar diseases are caused by *Rosellinia* and *Xylaria* among the ascomycetes. Their host range is wide among the tropical tree crops which can be affected when planted on land immediately after bush clearance (Hillocks & Waller, 1997).

2.5 Types of root diseases

There are several fungi which can cause root diseases in tea. There are four common root diseases of tea in Sri Lanka: Red Root disease, Black Root disease, Charcoal Root disease and Brown Root disease. Between this four root diseases, the Red Root disease is the most serious and majoring of economic importance in Sri Lanka, while White Root disease is of minor importance in tea (Mukerji & Bhasin, 1986).

There are several differences between these four root rot diseases. The red root disease is known as *Poria Hypolateritia*. This disease has been a common root disease in Sri Lanka since the first planting of tea on a plantation scale. For this root disease, the manner of the death of bush is gradually or entirely. The external appearance of root is red to black mycelial sheath or strands with white specks and there are soil particles adhere to their surface. While after the removal of bark, sometimes white patches of mycelium overlying the wood appear. The internal appearance of rot are the wood permeated by red lines or sheets, soft and it is getting moist when becoming old, (Wilson & Clifford, 1992).

The external appearance of root for the Black Root disease (*Rosellinia arcuata*) is roots that have been covered with a loose network of black and woolly mycelium. If the bark is removed the surface of the wood is found to be covered by white star-shaped patches of mycelium. In contrast, the appearance of Brown Root disease (*Fomes noxius*) is a root encrusted with soil particles and brown mycelium intermixed. Usually, after the removal of bark, scattered patches of white brownish mycelium can be seen. The wood is usually not badly decayed, but it is permeated

with yellow-brown sheets of mycelium which assumes a honey-comb structure and appears as a irregular brown lines when the wood is cut. For Charcoal root disease (*Ustulina deusta*), there are no visible symptoms on the surface. When the bark is removed the surface of the wood is found to be covered by white fan-shaped patches of mycelium. While when the wood is cut, the wood is permeated by irregular, single or double black lines, (Wilson & Clifford, 1992).

2.6 *Poria* disease

In Sabah Tea Plantation, the causal agent of the disease that has been attacking the plantation is thought to be *Poria Hypolateritia*. From the history, *poria* disease is undoubtedly a legacy from the jungles (Personal Communication: Warren, 2004). They come from the jungle where the fungus occurred, not as a virulent or a danger parasite but as an opportunistic species that has to struggle for its survival on such material as it could feed on. *Poria* disease occurs principally in tea grown on land which was originally jungle and it is rarely found in grassland clearings. After the jungle clearings, the *poria* disease first occurs in the neighborhood of a decaying jungle stump. This factor is the most frequent source of *poria's* spreading. The stumps have disappeared but the *poria* disease still remains in the soil. The fungus survived as a saprophyte on decaying roots after the jungle has been cleared. It survived as a saprophyte on decaying roots which served as a food base from. Besides that, this fungus can send out hyphae through the soil in search of more food. Those hyphae can penetrate into the roots of jungle trees but they can also penetrate the tea roots. No matter in what direction the hyphae grew, some would be sure to encounter the tea roots. Actually, the clearing of the jungle was beneficial to the fungus. The fungus

gets a benefit from this change because when the penetration into the tea was achieved, they took up a parasitic mode of life. The fungus also became independent of the original food base. Then, from here the fungus started to spread by using the infected roots of one bush as a food base from which to reach the next (Personal Communication: Warren, 2004).

The current name of *Poria hypolateritia* is *Tyromyces hypolateritius* (Anon, 2005 e). It is classified under phylum Basidiomycota and family of Polyporaceae (Alexopoulos, et al., 1996). The pathogen is inseparable and without distinctive taste; margin whitish to pale yellowish and more or less myceliod. Hyphal system apparently monomitic, the context of generative hyphae which are infrequently branched with thin to thick walls and inconspicuously nodose-sepatate (Anon, 2005 e). The hyphae are rarely branched and thick-walled. The cystidia variously shaped and frequently with a globose head (Ryvarden & Johansen, 1980).

The validity of this species is questionable. It is different from *Poria versipora* (*P.versipora*) that only in the more decidedly cartilaginous nature of the trama, which maybe simply a product of the age when collected or the way in which the specimen was dried. The types of *P. adpressa*, *P. eyrei* and *P. hypolateritia* have badly contaminated hymenia and the spore records are not wholly certain (Anon, 2005 e). Cultural work to test the validity of the distinction is highly desirable.

The fruit body of *Tyromyces* is resupinate, effused, adnate, soft when it is fresh, drying firm and subcartilaginous. Pore surface cream drying slightly darker and with a pale sordid of greenish tint (Ryvarden & Johansen, 1980). It is glancing when

REFERENCES

Agrios, G. N., 1997. Plant Pathology, 4th Ed, Academic Press, United States of America.

Alexopoulos, C.J., Mims, C.W. and Blackwell, M., 1996. *Introductory Mycology*, 4th Ed, John Wiley & Sons, Inc., Unites States of America.

Ann, P.J., 2002. *Phellinus noxius* brown root rot of fruit and ornamental trees in Taiwan. *Plant Disease*, 1-5.

Anon, 2004 a (http://www. rainfall pattern in Ranau.htm). September 2004.

Anon, 2004b (http://weather.cnn.com/weather/forecast.jsp?locCode=MS056). September 2004.

Anon, 2004c (http://www.soil type.htm). September 2004.

Anon, 2004d (http://www.bfahfh.de/bfhpers/pdf/os_pub1.pdf). September 2004.

Anon, 2005e (Data from CBS Aphylloporales database: http://www.cbs.knaw.nl/scripts/Aphylloporales.dll/ShowName?Nr=74378). January 2005.

Atong, M. (2005), Personal Communication.

Barrow, J.R. and Aaltonen, R.E., 2001. Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by physiological activity dark septate fungi and the influence of host. *Mycorrhiza.* **11**, 199-205.

Blakeman, J.P. and Williamson, B., 1994. *Ecology of Plant Pathogens.* CAB International, United Kingdom.

Crocker, J. and Murray, P.G., 2003. *Molecular Biology in Cellular Pathology.* John Wiley & Sons Ltd., England.

Eden, T., 1976. *Tea*, 3rd Edition. Longman Group Limited, London.

FAO,2004(http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGP/AGPC/DOC/Counprof/Malaysia.htm) September 2004.

Fox, RTV, 1993. *Principles of diagnostic techniques in plant pathology.* CAB International, Wallingford.

Gamborg, O.L. and Phillips, G.C., 1995. *Plant cell, Tissue and Organ Culture: Fundamental Methods.* Springer Lab Manual, Germany.

Gilman, J.C., 1957. A Manual of Soil Fungi, 2nd Edition. Oxford & IBH Publishing Co., Calcutta, India.

Gubler, W.D., Baumgartner, K., Browne, G.T., Eskalen, A., Latham, S.R., Petit, E., and Bayramian, L.A., 2004. Root diseases of grapevines in California and their control. *Australian Plant Pathology*, **33**, 157-165.

Hawksworth, D.L., 1994. *The identification and characterization of pest organisms.* CAB INTERNATIONAL, United Kingdom.

Hillocks, R.J. and Waller, J.M., 1997. *Soil borne Disease of Tropical Crops.* CAB INTERNATIONAL, United Kingdom.

Hindley, J., 1983. *DNA sequencing*. Elsevier Biomedical Press, Netherlands.

Hodgson, E. and Smart, R.C., 2001. *Introduction to Biochemical Toxicology.* 3rd Ed, John Wiley & Sons, Inc., United States of America.

Howard, K.L. and Moore, R.T., 1970. Ultrastructure of oosporogenesis in *Saprolegnia terrestris*. *Botanical Gazette* **131**, 311-336.

Jones, D.G., 1998. *The Epidemiology of Plant Diseases*. Kluwer Academic Publishers, Great Britain.

Kenaga, C.B., 1974. *Principles of phytapathology*. 2nd edition, Balt Publishers, United States of America.

Khairy, (2004), Personal Communication.

Kristiansen, K.A., Taylor, D.L., Kjoller, R., Rasmussens, H.N. and Rosendahl, S., 2001. Identification of mycorrizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. *Molecular Ecology* **10**, 2089-2093

Laessoe, T. and Spooner, B.M., 1994. *Rosellinia* and *Asterocystis (Xylariaceae)*: new species and generic concepts. *Kew Bulletin*, **49**, 1-70.

Manners, J.G., 1982. *Principles of Plant Pathology*. Cambridge University Press, United Kingdom.

Martin, H., 1972. *Insecticide and Fungicide Handbook: For crop protection*. 4th Ed, Blackwell Scientific Publications, Great Britain.

Moore, R.T., 1994. Cytology and ultastructure. In: C.P. Kurtzman and J. Fell (eds), *The Yeasts, A taxonomic Study*, 4th edition. Elsevier, Amsterdam.

Mukerji, K.G. and Bhasin, J., 1986. *Plant Diseases of India: A Source Book*. Mcgraw-Hill Publishing, New Delhi.

Mullis, K.B., Ferray. F. and Gibbs. R.A., 1994. *The Polymerase Chain Reaction.* Birkhauser, United States of America.

Palti, J. and Katan, J., 1997. *Effect of Cultivation Practices and Cropping Systems on Soilborne Diseases.* CAB International, United Kingdom.

Ryvarden, L. and Johansen, I., 1980. A preliminary polypore flora of East Africa, 608.

Schilthuizen, M., 2004, unpublished manual, University Malaysia Sabah, 2004.

Schmidt, O. and Moreth, U., 1998. Characterization of Indoor Rot Fungi by RAPD Analysis. *Natural Sciences.* (52), 229-233.

Sivapalan, P., Kulasegaram, S. and Kathiravetpillai, A., 1986. *Handbook of Tea.* The Tea Research of Sri Lanka, Sri Lanka.

Strange, R.N., 1993. *Plant Disease Control: Towards environmentally acceptable methods.* Chapman & Hall, Great Britain.

Summer, S.A., 1990. (http://www.Biological degradation of soil_S A Summer, 1990). February 2005.

Waller, J.M. and Holderness, M., 1997. *Soilborne diseases of tropical crops; Beverage crops and palms.* CAB International, United Kingdom.

Warren, J. (2004), Personal Communication.

Willson, K.C. and Clifford, M.N., 1992. *Tea: Cultivation to consumption.* Chapman & Hall, Great Britain.

Yamou, J. (2004), Personal Communication.

http://www.sabahtourism.com/aboutus/strategy_products.asp). September 2004.