Characterization, epidemiology and control strategies for the anthracnose pathogen (*Colletotrichum* spp.) on cashew (*Anarcardium occidentale* L.) in Mozambique

by

Americo Uaciquete

Submitted in partial fulfillment of the requirements for the degree of Ph.D. in Plant Pathology

In the Faculty of Natural and Agricultural Sciences
Department of Microbiology and Plant Pathology
University of Pretoria

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Abstract

The first confirmation of the presence of *Colletotrichum gloeosporioides* Penz. on cashew in Mozambique was based on a combination of observed symptoms, isolation and identification using basic morphological and molecular techniques. Anthracnose is now the second most important in the country, after powdery mildew caused by *Oidium anacardii* Noack. The present thesis represents a broad overview of the disease in Mozambique. The main focus of this study was thus to gather scientific information on the relevance of this disease in the country and through experimentation, generate recommendations that help farmers and decision makers to mitigate the disease pressure.

The **specific objectives** of this study were as follows:

- Provide a distinctive description of anthracnose symptoms on leaves through host-pathogen interaction studies in the laboratory.
- Enhance current knowledge on the identity of Mozambican pathogen isolates, using DNA tools.
- Assess the current anthracnose management practices, both at nursery and field level with a view to formulate timely, local and adequate management strategies.
- Conduct experimental trials to select economically effective fungicides spraying programs for anthracnose disease management.

- Search for variability and germplasm tolerance among dwarf and common cashew plant populations in Mozambique.

By analyzing and integrating existing published literature on the subject, we successfully separated issues that concerned previously inaccessible information from those that reflect insufficient scientific knowledge. A survey was initiated to determine, the status of cashew anthracnose disease management practices in Mozambique. Subsequently, the information obtained was used to develop a national strategic framework for research and extension in the country.

Areas identified as gaps were aligned with the main goals of this thesis and include:

- Areas where scientific information lacked were identified.
- The symptoms of the disease on leaves were successfully and distinctively distinguished from other common leaf diseases that simultaneously occur in orchards.
- The pathogen isolates were identified using PCR techniques. The presence of *Colletotrichum acutatum* Simmonds was not confirmed at least not among the suspected and tested isolates.
- Knowledge on the epidemiology of the disease was generated and its application for more effective disease management was successfully applied.
- Effective fungicide applications and disease control programmes were developed for *Colletotrichum gloeosporioides* Penz..
- Appropriate nursery management strategies that reduce anthracnose disease development were developed.
- Variability in germplasm reaction to the disease was demonstrated and therefore tolerant and susceptible genotypes were identified.
- A technique for rapid and accurate evaluation of leaf anthracnose symptom grades was developed.

DECLARATION

I, the undersigned, hereby declare that this thesis, submitted for the degree of Doctor of Philosophy, Plant Pathology, is my own and original work except where acknowledged.
This work has not been submitted for a degree at any other tertiary institution.

Signature……..

Date……..

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May the ***almighty Lord*** bless us.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADPP	People to people Danish development Agency
ADRA	Adventist development and relief agency
AI	Active ingredient
ANOVA	Analysis of variance
CLUSA	United States of America cooperatives league
CRD	Completely randomized design
CV	Coefficient of variance
DNA	Deoxyribose nucleic acid
EC	Emulsifiable concentrate
e.g.	Example
IIAM	Agriculture research Institute of Mozambique
INCAJU	Cashew Promotion Institute of Mozambique
ISSR	Inter simple sequence repeats
LSD	Least significant difference
MT	Metical (Mozambican currency)
NGO	Non-governmental organization
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pH	Percentage of hydrogen ions
PMD	Powdery mildew disease
PROB.	Probability
RAPD	Random amplified polymorphic DNA
SC	Suspension Concentrate
SEM	Standard error mean
UP	University of Pretoria
USD	United states dollars
VCG	Vegetative compatibility group
WG	Water dispersible granules
WP	Wettable powder
WV	World Vision

CHAPTER ONE: GENERAL INTRODUCTION

Cashew (*Anacardium occidentale* L) taxonomy started many years ago, when the Indian natives of South America gave it a name (De Araujo and Da Silva, 1995; Ferrão, 1995; Barros *et al*., 2002). Scientifically accepted taxonomic description of the genus *Anacardium* is associated with a French naturalist of the XIVth century. A molecular detailed review of Anacardeaceae family was provided by Pell (2004). Further biosystematics to the species known today, was established by Baily in 1942 (Mitchell and Mori, 1987; De Araujo and Da Silva, 1995; Behrens, 1996). Variations within the species are currently further studied by molecular tools such as random amplified polymorphic DNA (RAPD) (Mneney *et al*., 1998) and inter simple sequence repeats (ISSR) (Archak *et al*., 2003, Lihong *et al*., 2005; Asolkar *et al*., 2011). Taken by explorers the cashew species adapted easily into a diversity of environments throughout the world (De Araujo and Da Silva, 1995; Behrens, 1996). Thus, the plant was soon found in many countries of South America, Africa and Asia (Milheiro and Evaristo, 1994; De Araujo and Da Silva, 1995, Ferrão, 1995; Behrens, 1996; Anon^{a}., 1999). Such a cosmopolitan distribution raised the issue of its center of origin (Ferrão, 1999) that was later clarified, by Mitchell and Mori, (1987) using DNA techniques, as North East Brazil.

From Northeast Brazil (Vivek *et al*. 2013), cashew was introduced essentially for environmental purposes: conservation of coastal sandy soils and wind break (Behrens, 1996; Barros *et al*., 2002). Modern cashew importance has changed from natural to industrial applications. It extends from food to metallurgic and medicinal industries (Bisanda, 1998; Mbulanya, 1998; Nayar, 1998; Augustin, 2001; Barros *et al*., 2001; Filgueiras and Alves, 2001; Pillai, 2001; Salam, 2001; Soman, 2001; Kanji *et al*., 2004). In any of the above circumstances, direct job creation and millions of dollars involved in transactions, explain the social value of cashew (Cardoso *et al*., 1999; Freire *et al*., 2002; Kanji *et al*., 2004). In Mozambique alone, financial gains are about 20 million USD/year and direct jobs created by the processing industry were more than 20 000 employees by the year 2010 (Anon, 2007).

Despite the good agro-ecological and economic insertion of cashew worldwide, many biotic and abiotic factors hinder its potential productivity. Some of the factors include, socio-political (civil war), economic (low farm prices), biological (high pressure of pests and

diseases), institutional (research and extension failure), cultural (bush fires) and ecological (droughts) (Milheiro and Evaristo, 1994; Anon, 1998; Anon[b]., 1999; Leite, 1999; Kanji *et al*., 2004). This dissertation addresses cashew anthracnose caused by *Colletotrichum gloeosporioides* Penz. (Penz.) & Sacc. (Ohler, 1979; Cardoso and Freire, 2002) being the second most important biological limiting factor in Mozambique after powdery mildew (*Oidium anacardii* Noack) (Dhindsa and Monjana, 1984; Uaciquete, 2004).

The direct damage of *Colletotrichum gloeosporioides* to the host has been discussed by many authors (Ohler, 1979; Ponte, 1984; Milheiro and Evaristo, 1994; Ikisan, 2000; Cardoso and Freire, 2002; Freire and Cardoso, 2003). The economic impact of anthracnose on yield includes: dropping of flowers and young fruits or dieback of shoots and seedlings (Milheiro and Evaristo, 1994, Oliveira, 2002). It is known for instance that under severe circumstances, more than 50% seedling mortality may occur (Ponte, 1984).

Some key findings reported in the literature include:

- *Colletotrichum* species is a taxonomic group that is morphologically and genetically complex and vast (Muniz, 1998; Ivey *et al*., 2004). Thus, different techniques have been used to characterize *Colletotrichum* species: Molecular analysis includes Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Fragment Polymorphism (RFLP) of ribosomal DNA (rDNA) and mitochondrial DNA (mDNA), sequencing of conserved regions such as Internal Transcribed Spacers (ITS) and Internal Spacer Region (IGS) of rDNA, Amplified Fragment Length Polymorphysm (AFLP) and microssatelites (Armesto, 2013). Only a few studies have been correlated with pathogenicity. Additionally, existing cashew-*Colletotrichum* knowledge t directly apply to disease management strategies by small farmers. This suggests that further research on cashew *Colletotrichum* diversity would have to focus on adequate disease control approaches as with many other tree crops.

- Various authors have described anthracnose symptoms on cashew (Ohler, 1979; Milheiro and Evaristo, 1994; De Araujo and Da Silva, 1995; Lihong *et al*., 2005, Lakshmi *et al*., 2013). However, host genotype and environment related symptom variations have not been highlighted. Furthermore, the complexity usually caused by

the simultaneous infection with other pathogens like *Pestalotia* sp. on the same tissue (Freire and Cardoso, 2003;) and the resemblance with other diseases like the recently described blight, (*Cryptosporiopsis* sp.) (Sijaona *et al*. 2005) is still unclear. Therefore, the nature of symptoms observed under different circumstances might require a profound clarification if proper disease diagnosis is to be made.

- Most of the available information on cashew anthracnose epidemics was generated in Brazil, the country of origin of cashew. In fact, little has been published from other countries compared to the vast amount of publications dealing with other tropical fruit crops. Therefore, a hypothesis that there have been no anthracnose epidemics on cashew can be raised which is probably due to environmental variations or host diversity restrictions. However, anthracnose disease occurs in all cashew producing countries (Ohler, 1979; Ponte, 1984) and numerous techniques for disease assessment are available (Freire *et al*., 2002; Freire and Cardoso, 2003). An important aspect is that qualified human resources and funding has been insufficient in developing countries where cashew is primarily produced.

- Disease management strategies for *C. gloeosporioides* can be achieved through a combination of various methods (Ponte, 1984; Freire and Cardoso, 2003). Host resistance and chemical control is the most successful so far (Casulli, 1975; Cardoso *et al*., 1999; Freire *et al*., 2002). The use of scientifically proven cashew tolerant cultivars in East Africa has also not been attained.

- Although, fungicides may be adopted based on international recommendations, local refinements that includes testing of doses and frequencies and economic studies are generally required as per the local socio-economic and environmental conditions. Registed products are also not readily available.

- There is a current global trend to increase orchard planting areas and trans-boundary movement of dwarf and highly productive cashew genotypes. But these materials are known to be highly susceptible to anthracnose. Therefore an increased awareness on the value of integrated crop management has driven research towards localized studies on epidemiology and control of cashew anthracnose in East Africa.

Thus**,** the **main objective** of this study was to generate scientific information on the current status of anthracnose in Mozambique and subsequently use this information to plan for additional experiments and/or formulate project driven solutions to provide recommendations to farmers and decision makers both at nurseries and field plantation level.

The s**pecific objectives** of this thesis were as follows:

- Provide a distinct description of anthracnose symptoms on leaves through host-pathogen interaction studies in the laboratory.
- Enhance current knowledge on the identity of Mozambican pathogen isolates, using DNA tools.
- Assess the current anthracnose management practices, both at nursery and field level with a view to formulate timely, local and adequate management strategies.
- Conduct trials to select biological and economically effective fungicide spraying programmes for anthracnose disease management.
- Search for variability and germplasm tolerance among dwarf and common cashew populations in Mozambique.

To achieve the objectives above, a literature review (CHAPTER II) and survey (CHAPTER III) were done to assess the status of cashew anthracnose management practices in Mozambique. Un-clarified aspects of the disease distinctiveness on the leaves (CHAPTER IV) as well as pathogen identification by PCR technique (CHAPTER VII) were addressed through laboratory studies.

To cover the objective on development of suitable disease management strategies, various trials were conducted:

- At the nursery in Nampula, potted seedlings were rearranged to change the microclimate to favour the disease (CHAPTER V) and seedlings were categorized in terms of phenlogical age related sensitivity to the pathogen (CHAPTER V).
- Laboratory screening of fungicides was conducted (CHAPTER IV) and a field trial on effective and economic fungicide spraying programmes against anthracnose and powdery mildew was also conducted (CHAPTER VI)

- Difficulties in rapidly and accurately assessing the disease required innovative approaches. Thus, incidence and severity relationships were explored for the first time on cashew anthracnose system (CHAPTER VII) to understand the epidemiology of the disease over multiple locations and crop seasons. The incidence/severity approach was then applied to screen cashew genotypes for resistance (CHAPTER VIII).
- Finally, a summary of contributions and an overview of considerations are given in CHAPTER IX.

References

Anon. 1998. Componente Produção. Plano Director do Caju. Instituto de Fomento do Caju, INCAJU, Ministério da Agricultura, Maputo. 62 p.

Anon[a]. 1999. Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew (*Anacardium occidentale* L.). Pages 112-116 *in*: Summary reports of European commission supported STD-3 projects (1992-1995). CTA. [online http://intership.cta.int/pubs/std/vol2/pdf/221/pdf] 111-116, March 25, 2009.

Anon[b]. 1999. Sector do Caju. Resumo da estrategia de producao do caju e analise de algumas medidas de politica. INCAJU, Ministério da Agricultura, Maputo. 73 p.

Anon. 2007. Reuniao Anual da African Cashew Alliance (ACA). Subsector do Caju em Mocambique: Evolucao e Perspectivas. htt/www/africancashewalliance.com . March 23, 2008.

Archak, S., Gaikwad, A.B., Gautam, D., Rao, E.V.V.B., Swamy, K.R.M. & Karihaloo, J.L. 2003. DNA fingerprinting of Indian cashew (Anacardium occidentale L.) varieties using RAPD and ISSR techniques. *Euphytica 230:397-404*.

Armesto, C. 2013. Variabilidade biologica e molecular de Colletotrichum gloeosporioides em Cafeeiro. Ph D thesis. Universidade Federal de Lavras-UFLA. 101p.

Asolkar, T., Desai, A.R. & Singh, N.P. 2011. Molecular analysis of cashew genotypes and their half-sib progeny using RAPD marker. *Research Article, Biotecnol, Bioinf. Bioeng. 1: 495-504*.

Augustin, A. 2001. Utilization of cashew apple. Pages 57-66 *in*: Cashew the Millennium Nut. World Cashew Congress, 23-25 February. Souvenir. International Convention Centre, Kochi.

Barros, L.M., Paiva, J.R. & Cavalcanti, J.J.V. 2001. Cashew (*Anacardium occidentale* L.): A review of Brazilian current research situation. Pages 47-51, *in*: Cashew, the Millennium Nut. World Cashew Congress 23-25 February. International Convention Centre. Kochi.

Barros, L. M., Paiva, J.R., Crisótomo, J. R. & Cavalcante, J.J.V. 2002. Botânica, origem e distribuição geografica. Pages 18-20 *in*: Barros (ed.). Produção, produção e aspectos técnicos. Embrapa. Brazil.

Behrens, R. 1996. Cashew as an Agro-forestry Crop. Prospects and Potentials. Tropical Agriculture 9. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) Gmbh, Eschborn. 83 p.

Bisanda, E.T.N. 1998. Cashew nut shell liquid (CNSL) resins as matrix in plant fibre reinforced composites. Pages 217-221, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International cashew and coconut conference 17-21 February, 1997. Biohybrids International Ltd. Reading.

Cardoso, J.E., Cavalcanti, J.J.V., Cavalcante, M. De J.B., Aragao, M. Do L. & Felipe, E.M. 1999. Genetic resistance of dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mould and angular leaf spot. *Crop Protection 18:23-27.*

Cardoso, J.E. & Freire, F.C.O. 2002. Identificacao e manejo das principais doencas. Pages 41-56 in: Caju fitossanidade. Melo, Q. M.S. (ed.), Embrapa Informacao Tecnologica. Brasilia.

De Araujo, J. & Da Silva, V.V. 1995. Cajucultura. Modernas Tecnicas de Produção. Ministerio da Agricultura, do Abastecimento e da Reforma Agraria. Empresa Brasileira de Pesquisa Agropecuaria - EMBRAPA. Centro Nacional de Agroindustria tropical-CNTP, Fortaleza. 292 p.

Dhindsa, P.P. & Monjana, A.M. 1984. Index of plant diseases and associated organisms of Mozambique. *Tropical Pest Management 30:407-429.*

Ferrão, J.E.M. 1995. O cajueiro (*Anacardium occidentale* L.). Ministerio do planeamento e da administracao do territorio. Secretaria de Estado da Ciencia e Tecnologia. Instituto de Investigação Cientifica Tropical. Lisboa. 298 p.

Ferrão, J.E.M. 1999. Fruticultura Tropical: Especies com frutos comestiveis -Volume I. Instituto de Investigacao Cientifica Tropical. Missao de Macau. Lisboa. 621 p.

Filgueiras, H.A.C. & Alves, R.E. 2001. Cashew apple for fresh consumption in Brazil. Pages 53-55 *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Freire, F.C.O. & Cardoso, J.E. 2003. Doencas do cajueiro. Pages 192-225 in: Doencas de Fruteiras tropicais de interesse agroindustrial. Freire, F.C.O., Cardoso, J.E., Viana, F.M.P. (eds.). Embrapa Informacao Tecnica. Brasilia.

Freire, F.C.O., Cardoso, J.E., Dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21:489-494*

Ikisan, 2000. Cashew. Crop Information. http://www.Ikisan.com. January 27, 2008

Kanji, N., Vijfhuizen, C., Artur, L. & Braga, C. 2004. Liberalisation, gender, and livelihoods: The Mozambique cashew nut case. Summary report. International Institute for Environment and Development. Nederlanden. 35 p.

Lakshmi, B.K.M., Reddy, P.N. & Prasad, R.D. 2013. Cross-infection potential of *Colletotrichum gloeosporioides* Penz. Isolates causing anthracnose in subtropical fruit crops. Tropical Agriculture Research 22:183-193.

Leite, J.P. 1999. A guerra do caju e as relações Moçambique-India na época pos-colonial. Documentos de traballho no. 57, CesA. Lisboa.

Lihong, L., Zetan, C., Chenghai, C. & Congfa, L. 2005. Curso de Formação China-Moçambique sobre o cultivo do cajueiro. Tecnologia do Cultivo do Cajueiro. Ministerio da Agricultura da Republica Popular da China. Beijing. 73 p.

Mbulanya, G.G.N. 1998. The wear characteristics of frictional elements based on cashew nut shell liquid. Pages 206-210, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. and Mpunami, A.A. (eds). Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do cajueiro. Cultivar. Associação de Tecnicos de Culturas Tropicais, Porto. 204 p.

Mitchell, J.D. & Mori, S.A. 1987. The cashew and its relatives (*Anacardium: Anacardiaceae*). The New York Botanical Garden. New York. 76 p.

Mneney, E.E., Mantell, S.H., Tsoktouridis, G., Amin, S., Bessa, A.M.S. & Thangavelu, M. 1998. RAPD-Profiling of Tanzanian cashew (*Anacardium occidentale L.*). Pages 108-115, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. and Mpunami, A.A. (eds). Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Nayar, K.G. 1998. Cashew: A versatile nut for health. Pages 195-200 *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Ohler, J.G. 1979. Cashew. Communication no 71. Department of Agricultural Research of the Royal Tropical Institute, Amsterdam. 260 p.

Oliveira, V.H. 2002. Sistemas de Producao 1. Cultivo do Cajueiro Anao Precoce. Embrapa-Agroindustria Tropical. Fortaleza. 40 p.

Pell, S.K. 2004. Molecular systematics of the cashew family (Anacardeaceae). Ph.D. thesis. Louisiana State University. 144 p.

Pillai, P.G. 2001. Cashew nuts. Nutritious snacks that naturally taste great. Pages 73-77, *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Ponte, J.J. 1984. Doencas do cajueiro no Nordeste Brasileiro. Documento 10. EMBRAPA-Departamento de Difusao de Tecnologia. Brasilia. 51 p

Salam, M. A. 2001. Cashew research and development, Indian scenario, Pages 31-41 *in*: Cashew the millennium nut. World cashew congress, 23-25 February. Souvenir. International Convention Centre. Kochi.

Serra, I.M.R.S., Menezes, M., Coelho, R.S.B., Ferraz, G.M.G., Montarroyos, A.V.V. & Martins, L.S.S. 2011. Molecular analysis in the differentiation of *Colletotrichum gloeosporioides* isolates from cashew and mango trees. *Braz. Arch. Biol. Technol. 54:1099-1108.*

Simon, C.R. 2001. Cashew nut as a constituent of healthy diet. Pages 67-72 *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Uaciquete, A. 2004. Epidemiology and control of powdery mildew (*Oidium anacardii* Noack) on cashew (*Anacardium occidentale* L.) in Mozambique. M. Sc. (Plant Pathology) thesis. University of Pretoria, Pretoria.

Vivek, M.N., Manasa, M., Pallavi, S., Sachidananda Swamy, H.C. & Prashith Kekuda, T.R. 2013. Antibacterial potential of cashew apple (*Anacardium occidentale* L.) juice against clinical isolates of *Staphylacoccus aureaus* and *Staphylacoccus mutans*. Short communication. *Science, Technology and Arts Research Journal 2:144-146*.

CHAPTER TWO: LITERATURE REVIEW

2.1. The cashew crop

2.1.1. Origin, taxonomy and diversity

The cashew tree (*Anacardium occidentale* L.) is native to South America (Ohler, 1979; Barros *et al*., 2002) and Brazil is the country of origin (Mitchell and Mori, 1987; Behrens, 1996; Salam, 2001; Oliveira, 2002). The first published description of the genus *Anacardium* and its uses was provided by Andre Thevet in 1558 (Mitchell and Mori, 1987; Behrens, 1996). Later, Bailey in (1942), established the taxonomic position of the genus *Anacardium* as follows (De Araujo and Da Silva, 1995): Division IV: Spermatophyta; Subdivision II: Angiospermae; Class II: Dicotyledoneae; Sub-class I: Archichlamideae; Order 39: Sapindales; Family: Anacardiaceae.

The family Anacardiaceae comprises 60 to 74 genera and 400 to 600 species (Ohler, 1979; Mitchell and Mori, 1987; Barros *et al*., 2002; Pell, 2004). Members of this family are trees or shrubs that exudate resinous substances and grow abundantly in the tropics (Ohler, 1979; Barros *et al*., 2002). In addition to cashew, well known relatives within the Anacardiaceae family are the ornamental and fruit trees like sumac (*Rhus vernix* L.), pistachio (*Pistachia vera* L.), mango (*Mangifera indica* L.) and African plum (*Sclerocarya birrea* (A. Rich.) Aubr.) (De Araujo and Da Silva, 1995; Behrens, 1996; Shomari and Topper, 2003).

Analysis of variability patterns within the genus *Anacardium* revealed a large variability of botanical and agronomic characters of agro-industrial interest (De Araujo and Da Silva, 1995). Within this species, concepts such as clone, line, variety and type have been explored by researchers to highlight specific qualities of interest in the selection and breeding processes (Mitchell and Mori, 1987; Gunjate and Patwardhan, 1995; Behrens, 1996; Barros *et al*., 2001; Salam, 2001; Lihong *et al*., 2005). However, so far there is no sufficient basis for description and identification of true varieties as per the current body of knowledge published in botanical, horticultural or other scientific journals (Lihong *et al*., 2005). Traditionally, in Benin for example, several vaieties have been recognized based on plant morphotypes or nut and apple characteristics (Tandjiekpon, 2005; Chabi *et al*., 2013;). Thus, despite some work

done in Tanzania (Mneney *et al*., 1998), only a few researchers, have established genetic relationships between cashew populations, based on biological molecular techniques such as random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) (Archak *et al*., 2003; Samal *et al*., 2003; Lihong *et al*., 2005; Asolkar *et al*., 2011).

2.1.2. Growing pattern

The extensive root system consists of one central taproot and a complex of extensive lateral roots (Anon. 1992; Milheiro and Evaristo, 1994). Lateral roots in adult plants may extend to almost 20 meters from the trunk (Barros, 2002). This complex root structure allows the plant to withstand long periods of drought during growth (Lopes et al., 1993). Cashew vegetative growth is continuous during the first 15 years (Milheiro and Evaristo, 1994). But once the plant has reached a full bearing stage (between 10 and 15 years) it sequentially undergoes a vegetative flush and generative flower flush annually (Hartman *et al*., 1981; De Araujo and Da Silva, 1995; Ferrão, 1995). The leaves are simple, alternate, obovate, glabrous, peninerved, up to 20 cm long and 15 cm wide, apically rounded or notched, entire, with a short petiole (Duke, 1989), stomata are encountered on both surfaces (Mitchell and Mori, 1987) and foliar nectarines present on leaf blades (Rickson and Rickson, 1998).

The inflorescence consists of a terminal panicle, 10-20 cm long, which is either sparse or congested (Mitchell and Mori, 1987; Duke, 1989). The panicle consists of both hermaphroditic and unisexual male flowers (Lopes *et al*., 1993). The ratio between male and bisexual flowers varies from 2:1 to 200:1 (Milheiro and Evaristo, 1994). Of the hermaphrodite flowers, only 10 to 19% produce mature fruits (Ferrão, 1995; Behrens, 1996). Thus, on average, five to six fruits develop to maturity in each panicle (Ferrão, 1995) although more than 15 mature fruits per panicle have been registered (Ohler, 1979). However, productivity of individual trees is determined not by number of fruits per panicle but essentially by the number of panicles per tree (Milheiro and Evaristo, 1994) which varies from 100-400 depending more on the size of the tree than on genetic characteristics (Behrens, 1996).

Nair *et al*. (1979) noted that flowering appears in two or three distinct phases. The duration of the whole generative flush process is staggered and continues for 52-125 days with the mixed

phase being the most prolonged (Nair *et al*., 1979; Wild, 1994; Gunjate and Patwardhan, 1995; Behrens, 1996). However, Barros *et al.* (2002) and Behrens, (1996) noted that the period of flowering initiation and duration, over the age of the plant or season in a year, varies with the genotype and environment. Therefore a traditional heterozygous plantation expresses mixed phenological stages (Freire *et al*., 2002).

2.1.3. Importance and production

The importance of cashew has been viewed in single or multiple perspectives:

- The vast and ever growing number of countries (Fig. 2.1) and hectares covered by the crop expresses the indirect economic and ecological value. Thus, in Asia, cashew is cultivated in India, Vietnam, Sri-Lanka, Indonesia, Philippines, Malaysia, Thailand and others. In Africa, cashew is cultivated in Mozambique, Nigeria, Kenya, Tanzania, Madagascar, Senegal, Benin, Ivory Coast, Angola, Guine Bissau, Uganda, etc. In the Americas, it is found in Brazil, El Salvador, Venezuela and Dominican Republic (Milheiro and Evaristo, 1994; De Araujo and Da Silva, 1995; Ferrão, 1995; Nair, 2001; Freire *et al*., 2002; Topper and Caligari, 2003). In general cashew production extends through the tropical areas of the Atlantic and Indian oceans coastal zones (Milheiro and Evaristo, 1994; De Araujo and Da Silva, 1995) and the global cashew area has been estimated at 26.0 million of hectares (Salam, 2001).

- The vast number of natural and industrial uses of cashew products and sub-products is elucidative of the crop value. The cashew apple has been demonstrated to possess antimicrobial and antioxidant activities (Gordon *et al*., 2012; Daramola, 2013; Manasa *et al*., 2013).

- Besides the nuts, the apples are used for producing alcoholic beverages, juices, etc.

- The cashew nut shell liquid is valuable in the metallurgic industry, the fruits and other parts of the plant have direct or indirect medicinal applications (Silva, 1961; Behrens, 1996; Bisanda, 1998; Mbulanya, 1998; Nayar, 1998; Augustin, 2001; Barros *et al*.,

2001; Filgueiras and Alves, 2001; Simon, 2001; Kanji *et al*., 2004; Amorati *et al*., 2011; Mazzetto *et al*., 2012).

- The importance of cashew is also viewed by the number of job opportunities generated throughout the whole crop chain from production, commercialization of raw nuts or apples, processing and marketing of products and derivatives (Cardoso *et al*., 1999; Freire *et al*., 2002; Kanji *et al*., 2004). In Brazil, in one season only, cashew provided 16 000 jobs in nut processing, 43 000 jobs in farm management and 280 000 temporary jobs during the harvest period (Barros *et al*., 2002). Kumar (1995) calculated that there are 30 000 potential employment opportunities lost to the various cashew producing countries in Africa from not processing raw nuts within the respective countries. In Mozambique the current scenario and projections are encouraging (Table 2.1). Over 1.5 million rural households are reported to derive their annual income mainly from cashew (INE, 2011)

- Cashew value can be better expressed in monetary terms from the individual farmers to countries, continents and the entire world. (Behrens, 1996; Mosha *et al*., 1998; Rao, 1998; Cardoso *et al*., 1999; Freire *et al*., 2002; Nathaniel *et al*., 2003). Kumar (1995) estimated that the cashew industry worldwide is worth USD500 million of which USD0.25/kg are for the farmer, USD1.30/kg for the processor and USD3.5/kg for retailer. In global trade for major edible nuts, cashew ranks second or third (Menge[a] *et al*., 2011). FAO statistics indicate that about 1 500 million Metrics tones of raw cashew nuts were produced in 2012 and valued for 1.5 bilions of dolares (FAOSTAT, 2012). In Brazil, the annual turn-over only from cashew kernel export has been estimated ranging between 200 and 230 million dollars (Barros *et al*., 2001; Freire *et al*., 2002; Lopez and Lucas, 2010). In Mozambique current financial gains are about 20 millions of USD/year (Anon., 2007).

- Additionally, the importance of the crop can be approached by positioning the cashew nuts in relation to other nuts such as almonds, walnuts, peanuts, coconuts, etc. or against other tree crops or food products. Topper and Caligari (2003) noted that the demand of cashew nuts in international markets was consistently higher when compared to other tree crops products such as coffee and cocoa. Augustin (2001)

compared the chemical composition of cashew apple with that of citrus fruit (lemon) while Simon (2001) compared the cashew nut nutrient values with selected animal foods such as fish and chicken. Finally Pillai (2001) highlighted the absence of cholesterol and sodium from the cashew nuts to emphasize its healthy nature. In terms of foreign currency generation in Mozambique cashew is ranked among the three major agricultural products that is alongside sugarcane and cotton.

- Another manner of valuing cashew is by placing it among the luxury products and therefore consumed in developed countries. This perspective provides emphases on the destinations of cashew kernels and thus developed countries such as EUA, Canada, France, Japan, Netherlands, Russia, United Kingdom, and Germany are the main importers (Milheiro and Evaristo, 1994; De Araujo and Da Silva, 1995, Ferrao, 1995; Behrens, 1996; Anon, 1998; Nair, 2007).

- A final and more recent approach consists in assessing the relative weight of a product towards poverty alleviation. As detailed by Walker *et al*. (2006), the method integrates the quantifiable value of individual produce into a series of equations that assess the magnitude of poverty at family level. In this context, studying the situation in Mozambique, Walker *et al*., (2006), observed that among all agriculture products including livestock, cashew ranked five in terms of its contribution toward reduction of rural poverty.

The various approaches presented previously are within the scope of international importance of cashew. In Mozambique, its value comes from the XVIth century history when Portuguese travelers introduced the plant into East African coastal zones initially to control erosion and provide shade (Ohler, 1979; Milheiro and Evaristo, 1994; Ferrão; 1995; Ferrão, 1999). Over time, the crop has been associated with global changes so that by 1945 (end of world war II), massive plantations of cashew were established (Appendix 1) as an economically strategic crop and the first export of raw nuts to India took place in 1955 (Leite, 1999; Anon., 2007).

Since then, the interest in the crop grew and today authors such as Milheiro and Evaristo, 1994; Leite, 1999 and Kanji *et al*., 2004, have discussed the methods and uses of cashew in Mozambique in both historical and technological horizons (Ferrão, 1995; Anon., 1999; Anon.,

2007). Nevertheless, till today only three cashew products, namely raw nuts, kernels and cashew nut shell liquid are used to generate foreign currency through export. Most of the juicy apples are wasted un-used although some are used in traditional preparation of juice, wine and spirits for domestic consumption or direct animal feed.

Due to its importance, an ever growing cashew production tendency at global, regional and country levels has been observed world-wide (Nair, 2007). Using data of year 2001, the relative position of Mozambique in the global scenario of cashew production has been analyzed in different perspectives such as area under cultivation, total production over time, and productivity (Salam, 2006). An evident aspect from the data is the high productivity of the Mozambican cashew trees, comparable to India and Brazil where there application of inputs has been commonly adopted. Mozambican cashew production may be attributed to agro-ecological suitability of the country for the crop and little management without inputs.

Contrasting with the adequacy of the environment for cashew, the investment has been minor. Based on production public investments data presented by Anon (1998) added to approximately 10 million USD from direct projects, it is evident that only 27 USD were allocated per cashew farmer or roughly one dollar per individual cashew tree during the last five years.

In addition to the small financial investment made, major historic events have drastically impacted on the cashew industry in Mozambique and consequently two consecutive crises were experienced (Fig. 2.2).

The first crisis is observed when the world cashew production record obtained in 1973 (Fig. 2.3) could not be sustained after independence and so it dropped to about 18000 tons. Many reasons have been attributed including socio-political (civil war and consequent displacement of cashew farming communities), economic (low farm prices, inaccessible financial credit and weakened rural trade networks), biological (high pressure of pests and diseases), institutional (weakened supporting research and extension services), cultural (uncontrolled bush fires associated with hunting behavior), ecological (frequency of drought periods) and the aging of the trees (Milheiro and Evaristo, 1994; Anon., 1998; Anon[b]., 1999; Leite, 1999; Kanji *et al*., 2004).

The second crisis also known as the industrial crisis emerged when the government liberalized the cashew market. This was under the assumption that financial gains at farmers' level would increase, since the competition among buyers could lead to unit price increase and thus encourage planting and better management. In fact, it occurred in the neighbouring Tanzania (Topper and Caligari, 2003). Disappointingly, in Mozambique, the prices did not increase and the national processing industry could not compete enough for raw nuts at farmers' gate and thus many factories closed. More than 10000 workers lost their jobs (Leite, 1999; Kanji *et al*., 2004).

In 1997, yet under the crisis scenario, the government created a new cashew institute (INCAJU) with a mandate of reviving the whole cashew chain from production, to commercialization of raw nuts, processing and export of kernels (Anon., 1997). Thus a master plan was developed through a critical analysis of the prevailing problems and transformed some into challenges (Cumbi, 1998; Anon[b]., 1999). Significant changes were implemented in a context of a range of opportunities such as adequate climatic and political will (Walker *et al*., 2006); secular experience of the farmers (Milheiro and Evaristo, 1994) and the availability of technology for integrated cashew management from the neighbor Tanzania (Kasuga *et al*., 2003). The international demand for cashew was still at high (Ascenso and Duncan, 1998, Shomari and Topper, 2003; Aksoy and Yagci, 2012), many trees, although old, were still alive and could be rehabilitated. Finally, land for new planting was also available (Anon[b], 1999) and many supportive NGO's operating in the cashew areas were ready to implement cashew activities (Anon, 1998).

Remarkably, the implementation of the master plan strategies triggered a new phase of cashew production growth trend (Fig. 2.4.) that led to new records of 104 000 tons and 113 000 tons in 2004/2005 and 2010/11 crop seasons respectively (Fig. 2.3). In addition to the income generated from the increased yield, the quality of nuts measured as out-turn increased from 42 to 44 units (Anon., 2006). Informal job creation related to fungicide spraying services was over 4500 positions in a five years period and more than 4 million grafted seedlings were produced. Four cashew clones were scientifically described and selected for registration and more importantly the present study was initiated.

2.2. The disease

2.2.1. Etiology

Cashew anthracnose is caused by *Colletotrichum gloeosporioides* Penz. (Penz.) & Sacc. (Ohler, 1979; Milheiro and Evaristo, 1994; Ferrão, 1995; Cardoso and Freire, 2002). It is a species group, variable in morphology, pathogenicity and physiology (izoenzymes produced) (Freire and Cardoso, 2003). In fact, over 1000 form-species of *Colletotrichum* have been described. However, on the later classification systems about 600 were found to be synonymous to *C. gloeosporioides* species group (Alexopoulos and Mims, 1979; Hawksworth *et al*., 1995). The species belonged to an anamorphic subdivision (Deuteromycotina), form-class of Coelomycetidae, order Melanconiales and family Melanconiaceae (Alexopoulos and Mims, 1979; Freire and Cardoso, 2003). With changes in classification system it has been modified quite recently so that the anamorph was integrated as Eukaryota, a fungi among filamentous ascomycetes that comprises the mitosporic group of Deuteromycetes (Agrios, 2005). Its teleomorphic form, *Glomerella cingulata* (Ston.) Spauld. & Shrenk (Milheiro and Evaristo, 1994; Ferrão, 1995; Cardoso and Freire, 2002) obtained only *in vitro* (Freire and Cardoso, 2003) belonged to class Ascomycetes, subclass Pyrenomycetes II and family Plystigmataceae (Alexopoulos and Mims, 1979). Now, according to Agrios (2005), the teleomorph is among the Pyrenomycetes of Order Phyllachorales. Because the teleomorph has not been found in nature it is deprived of any epidemic relevance (Cardoso and Freire, 2002).

Understanding of the morphological identity and genetic complexity of pathogen population infecting a particular host is important in designing effective disease management strategies (Swart, 1999; Agrios, 2005). The importance is even higher when one observes that pathogen variations are often dynamic and reflects a co-evolution with particular host species or cultivar (Manners *et al*., 1992) or they may result from significant environmental changes caused by nature or human activities. Development of resistant strains for instance may be induced by a continuous application of highly specific fungicides (Agrios, 2005) and therefore, different species of the genus *Colletotrichum* Corda, have been isolated occurring simultaneously on the same host, for instance *C. dematium, C. crassipes and C. acutatum* occur together on the rubber tree (Guyot *et al*., 2005). In West Africa a *C. gloeosporioides*

collection of isolates was established from different cashew organs, different geographic origins and even from other tropical fruit (Anon[a]., 1999). Furthermore, morphocultural and isoenzymatic characteristics have been used for the description of *C. gloeosporioides* isolates from cashew (Muniz *et al*., 1998; Anon[a], 1999; Freire *et al*., 2002) and evidence of variability was found.

In general, morphological and cultural characters commonly used to differentiate *Colletotrichum* species include conidial morphology, presence or absence of setae, presence or absence of teleomorph, colony color, pigment production, growth rate and appressorium features (Ivey *et al.*, 2004). These characteristics have been used for the descriptions of various isolates of cashew *C. gloeosporioides* (Muniz *et al*., 1998). However, because definitive identification of *Colletotrichum* species based on morphology is difficult where isolates have overlapping ranges of conidial and colony characteristics and variation in morphology is accepted for isolates within species (Mills *et al*., 1992; Ivey *et al*., 2004), a number of molecular methods have been used to characterize species of *Colletotrichum* (Mills *et al*., 1992; Ivey *et al*., 2004).

Another method used for characterization of *Colletotrichum* species and thus revealing diversity is pathogenicity testing. On one hand isolates from cashew have been found to infect mango (Piteira and Rodrigues, 1999) on the other hand cashew isolates of *Colletotrichum* have failed to infect mango and vice-versa (Freire *et al*., 2003). Recently, cross infection of isolates from both mango and cashew was established (Lakshmi and Prasad, 2011). These contradicting results may require further verification. However, cross-infection studies are affected by the interactive environment created under simulated field conditions as well as the host cultivars envolved. However, this does not necessarily constitute a threat under the field conditions (Swart, 1999). An additional variation on *C. gloeosporioides* was detected when pathogenicity tests were conducted on detached cashew leaves of a single clone. The results showed that isolates of *C. gloeosporioides* have differential virulence levels (Anon[a]., 1999).

Furthermore, vegetative compatibility groups (VCGs) have also been used to understand population diversity of *Colletotrichum* spp. and *G. cingulata* isolates. The frequencies of

VCGs suggested that they were location or cultivar specific (Gonzalez and Sutton, 2004). To our knowledge none of these methods has been applied on cashew so far.

2.2.2. Symptoms

The term anthracnose is derived from Latin language (anthrax = carbon= black) and refers to blackening diseases of foliage, stems or fruits that typically appear as dark-colored spots or sunken lesions with slightly raised rim (Agrios, 2005). These symptoms have been described from various tropical perennial and other cash crops (Waller, 1992).

On cashew, anthracnose (*C. gloeosporioides*) occurs in all aerial parts of a cashew tree (Ponte, 1984). However, the symptoms are more commonly observed on the leaves (Freire and Cardoso, 2002). Early symptoms are pale, developing into a reddish–brown, shiny and water soaked or oily irregular lesions sometimes with resin exudations (Ohler, 1979; Milheiro and Evaristo, 1994; Cardoso and Freire, 2003). The lesions are variable in size and may occur anywhere on the leaf limb, apex or edges (Ponte, 1984).

When the infection is severe, multiple individual spots may expand and coalesce to one another covering more than a half of the leaf from the apex downwards (Cardoso and Freire, 2002; Ponte, 1984). Once affected, tender tissues may cause irregular growth or crinkling, followed by a dry up and fall off with appearance of blight (Ponte, 1984; Ikisan, 2000). Sometimes, because the expansion of the lesions are delimited by mature tissues, with age, they simply crack and leaving a hole on the leaf limb (Ponte, 1984; Cardoso and Freire, 2002).

Characteristic acervuli of the fungus are massive and frequently formed on the adaxial and rarely on the abaxial necrotic leaf surface (Freire and Cardoso, 2003). The acervuli can be observed as small and elevated black dots on the leaf surface (Ponte, 1984). From these, numerous conidia are released by eruption (Freire and Cardoso, 2002).

On the panicle main stalk and lateral branches, anthracnose lesions are characteristically depressed and resinous, elliptical in shape, like on leaves, reddish-brown in color and radial and longitudinally expanded (Ohler, 1979; Ponte, 1984; Cardoso and Freire, 2002; Freire and

Cardoso, 2003). Such lesions may isolate the flowers from the source of nutrients with consequent withering, blackening and drop off and total crumpling of the whole inflorescence (Ohler, 1979; Ponte, 1984; Ikisan, 2000; Freire and Cardoso, 2003).

Immature fruits when infected turn black and fall prematurely (Ikisan, 2000) or on growth the apples develop some longitudinal cracks and mummifies or it eventually rots and fall (Ohler, 1979; Ponte, 1984; Milheiro and Evaristo, 1994; Freire and Cardoso, 2003). When mature fruits are infected, they develop black, depressed, irregular or roundish spots that are often sunken (Ikisan, 2000).

Infection on twigs shows initially purple and later dark-brown to black and depressed, elongated lesions that finally lead to regressive die-back (Ponte, 1984; Ikisan, 2000). Recurrent die-back of shoots may induce stag headed symptoms and subsequent death of the plant especially at growth young stages (Ohler, 1979; Milheiro and Evaristo, 1994).

Dropping of flowers and young fruits is the main and measurable damage of anthracnose disease on cashew yield (Oliveira, 2002) and shoots and seedlings die-back are responsible for indirect economic impact (Milheiro and Evaristo, 1994).

2.2.3. Epidemiology

Plant disease epidemic or epiphytotics is "when a pathogen spreads to affects many individuals within a relatively short time or simply the dynamic of change in plant disease in time and space" (Agrios, 2005). Comprehension of the factors that may interfere with the activity of the pathogen is important in controlling the disease, especially when forecasting models are adopted in order to time fungicide applications (Estrada *et al*., 2000) or when biological control approaches are to be adopted (Dodd *et al*., 1992).

Factors affecting *C. gloeosporioides* pathosystems are many and have been reviewed by Dodd *et al*., (1992). These include duration of leaf wetness, humidity, light or competitive microflora, temperature, rainfall intensity and duration, crop geometry, density and phenological patterns, mist or dew, wind intensity and direction (Dodd *et al*., 1992; Agrios, 2005). In many cases, pathogen isolates may have to adjust their cycle to these factors and

their dynamics (Dodd *et al*., 1992). Thus, on cashew infected organs *C. gloeosporioides* produces innumerous reproductive asexual structures called acervuli (Ponte, 1984; Cardoso and Freire, 2002; Freire and Cardoso, 2003) which is a form of conidiomata or multi-hyphal conidia bearing structure (Hawksworth *et al*., 1995).

Sutton (1966) noted that *Colletotrichum* species have two distinct types of acervuli: pulvinate, the most commonly found and hypostramatic acervulus which is characteristic of species that attack graminaceous plants. On cashew, the salmon-to-black colored acervuli are pulvinate and erupt through the cuticle by mechanical force. They are scattered and distanced from one another but frequently arranged in concentric rings on apples (Sutton, 1966, Ponte, 1984; Intini, 1987). The acervuli may or may not possess setae, depending on the organ in which they are formed: Those formed on leaves frequently possess setae while those formed on fruits will rarely produce setae (Ponte, 1984). However, it should be noted that this feature in the genus *Colletotrichum* is quite variable particularly under cultural conditions (Alexopoulos and Mims, 1979).

In the genus *Colletotrichum*, conidiogenisis or process of conidium formation (Hawksworth *et al*., 1995) involves a specialized hyphae referred to as a conidiophore (Agrios, 2005) and a terminal conidiogenous cell on which one or more conidia are produced (Hawksworth *et al*., 1995). Conidiogenous cells in this genus are enteroblastic, phialidic, hyaline to brown in color, smooth textured, cylindrical to sub-cylindrical. They can be integrated or discrete with conspicuous colarette and occasionally prominent periclinal thickening (Sutton, 1966).

Conidium means an asexual spore (Hawksworth *et al*., 1995). Numerous conidia are released from the phialides within the acervuli and they are ellipsoidal, hyaline and unicellular (Ponte, 1984; Intini, 1987; Cardoso and Freire, 2002). Like other members of the genus *Colletotrichum*, they are smooth in texture, slightly narrow in the middle than at the ends and become uni-septate prior to germination (Sutton, 1966; Alexopoulos and Mims, 1979).

Conidia are maintained in a gelatinous matrix that aggregates, protects and confers them auto-inhibition for germination (Freire and Cardoso, 2003). The involvement of this matrix on infection processes of various *Colletotrichum* pathosystems has been largely discussed

(Bailey *et al*., 1992; O'Connel *et al*., 1992) but the cashew pathosystem appears to be less studied.

For dissemination from one organ to another within the same plant (self-infection) or to a neighboring plant, conidia are carried by rain splashes or mist drippings or by wind-blown micro-droplets (alo-infection) (Ponte, 1984; Intini, 1987; Freire and Cardoso, 2003). Water related processes dissolve the conidial gelatinous matrix (Ponte, 1984; Cardoso and Freire, 2002). This explains why high incidence and severity of anthracnose are associated with water droplets from the rainfall (Cardoso *et al*., 1999; Ikisan, 2000). Conidia are most important in dissemination, host contact and pre-germination processes (Waller, 1992). Adhesion of *Colletotrichum* species onto host surface is non-specific (Bailey *et al*., 1992).

Once in contact with the new susceptible surface (shoots, leaves, inflorescences or fruits) disaggregated conidia, are dependable to relative humidity of air for germination (Dodd *et al*., 1991). Germination is inhibited at atmospheric relative humidity lower than 95% (Ohler, 1979). When the conditions are favorable, the conidia germinate by emitting a pro-mycelium or primary germ tube at the extremity of which an appressorium is formed in response to the host physical and chemical stimuli (Ponte, 1984; Freire and Cardoso, 2003). Most frequently, from individual conidia, a single germ tube emerges and from this, one appressorium is formed between 6 and 12 hours depending of the host species (Freire and Cardoso, 2003). From other host-pathogen interactions it is known that once the conidia adhered 12-48 hours later a germ tube emerges and grows for about 10-20 µm before swelling into a terminal appressorium (Bailey *et al*., 1992). Therefore on cashew, the emergency of appressoria is relatively fast. The role of the appressorium is to facilitate host penetration (Ponte, 1984). As the appressorium ages, it become darkly pigmented with melanin (Jeffries *et al*., 1990) a substance whose role in the process of infection has been discussed also from other hosts (Kubo *et al*., 1992, Howard and Ferrari, 1989).

On one hand, penetration can be direct, through the cuticle and cell wall (Ponte, 1984) by involving mechanical force in association with macerating enzymes from the cell wall structure (Freire and Cardoso, 2003). In this case, no injury or natural openings such as stomata are required (Ponte, 1984; Freire and Cardoso, 2003). On the other hand, like other diseases, *Helopeltis*, a common sucking pest, may inoculate the fungus through its tiny sting

by pricing into the host (Ohler, 1979; Intini, 1987). Both penetration mechanisms are well known from other *Colletotrichum* species and they were thoroughly revised by Bailey *et al*. (1992).

In addition to restrictions caused by lower relative humidity levels (Ohler, 1979), spore germination for *Colletotrichum* requires conditions of average temperatures not above 30°C (Ohler, 1979; Milheiro and Evaristo, 1994; Ferrão, 1995). The optimum temperatures for spore germination are between 22 and 29°C (Ponte, 1984; Freire *et al*., 2002). In fact, intermittent rains and consequent high humidity levels, combined with temperatures ranging between 24 and 32°C during the flowering and fruiting stages, are the most favorable conditions for anthracnose dispersion (Ikisan, 2000). In the north border neighboring Tanzania, adequate conditions for anthracnose, extends from May to September not due rains. It is mainly because during the night and early hours of the mornings, condensation of water on the receptive organs of the tree is sufficient to release and disaggregate the conidia from the acervuli and during the day, constant winds facilitate their dissemination (Intini, 1987).

When climatic conditions or cashew phenological stages become adverse for *Colletotrichum*, it either remains dormant on the lesions (Milheiro and Evaristo, 1994) or it survives saprophytically on death tissues pending on the host or on fallen debris (Ponte, 1984). Dormant mycelium returns to activity and subsequent production of new spores when sap circulation commences with emergence of new shoots (Milheiro and Evaristo, 1984). This hosts' triggered activation may represent an ecological adaptation of the pathogen to synchronize with abundance of receptive host tissues.

Other strategy for seasonal carryover from the pathogen, may involve survival on other hosts different from mango (*Mangifera indica* L). The assumption is because, *Colletotrichum* is a polifage parasite, that is, it has a wide spectrum of action and cross inoculation of pathotypes has proven not to infect mango and vice-verse (Ponte, 1984; Freire and Cardoso, 2003).

The other seasonal carryover strategy may involve active survival on off-season flashing plants from heterogeneous seed borne plantations (Freire *et al*., 2002) or on receptive organs of dwarf young and grafted seedlings with massive continuous growth (Sundararaju and

Babu, 1999; Cardoso *et al*., 2002). A full disease cycle model based on mango anthracnose is presented in (Fig. 2.5) and the calendar of cashew events is presented in appendix 2.

2.2.4. Distribution

The importance of knowledge on disease distribution is extensive to regional, national and even farm level. Information on disease mapping and intensity is applied for multiple purposes such as decision making for economic investment risk assessment and warning systems for epidemics, quarantine measures for trans-boundary movement of germplasm, etc. and many examples of successful use are available (Agrios, 2005).

The genus *Colletotrichum* occurs commonly in warm moist environments encountered in the humid and sub-humid tropical zones where it can often be isolated from either healthy or diseased tissues (Waller, 1992). Cashew anthracnose was recorded as such for the first time in Trinidad Tobago (Baker *et al*., 1940). Later, in Brazil the disease was called in Portuguese "Queima do cajueiro" (Da Matta & Lellis, 1973) a term that can be translated as "cashew blight". In India (Tamil Nadu), the first record of epidemic is dated 1965 and was called "soorai" (Nambiar, 1978) after the first official record of anthracnose in Mozambique by Carvalho and Mendes (1958). In Tanzania, official record was during 1960 but it was admitted that the disease may have been there long before (Casulli, 1975). Today, the disease also occurs in Malaysia (Lim and Singh, 1985) in Guinea Bissau, (Muniz *et al.*, 1998) and many other countries throughout the whole cashew growing areas (Intini, 1987) (Table 2.2).

Very few examples of national survey or mapping of cashew anthracnose disease are found in the literature. But a number of techniques to gather geographic information are known from other diseases or fruit trees and these depend on purpose for which the information is required, the cost, the urgency required, accumulated knowledge, etc. Agrios (2005) presented and analyzed the advantages and disadvantages of most of the techniques for example the molecular tools, geographic information systems, global positioning systems, geostatistics and remote sensing and image analysis just to mention the modern ones.

In Brazil, where qualitative information on the distribution of main cashew diseases was gathered, anthracnose was found occurring throughout the whole cashew area with relatively

high intensity than most other diseases (Ponte, 1994). Similarly, in Mozambique, cashew anthracnose was found to occur in all cashew provinces and placed as the second most important disease after cashew powdery mildew (*Oidium anacardii* Noack). The need for chemical intervention was also highlighted (Dhindsa and Monjana, 1984).

2.2.5. Disease Management

A primary prophylactic measure for anthracnose disease is to prevent overcrowding of trees in the orchard (Ikisan, 2000). This can be achieved through adoption of appropriate spacing which in turn depend on the type of cashew whether it is dwarf or common, cultivar and growth pattern (intensive or extensive), propagation technique (seed borne or grafted) and finally soil fertility and cultural measures adopted.

All the control measures today are essentially directed to reduce initial inoculum and or disease progress rates in the plantation (Cardoso and Freire, 2002; Freire and Cardoso, 2003). Thus, immediately after the rainy season all foci must be eliminated through pruning and burning of the infected parts (Ponte, 1984; Ikisan, 2000; Cardoso and Freire, 2002; Freire and Cardoso, 2003). During the establishment of new plantations only healthy seedlings must be used (Freire and Cardoso, 1995). Cultural and biological measures in *Colletotrichum* pathological systems of perennial plants have been discussed (Waller, 1992). The control options to be implemented on cashew would have to take into consideration the complexity of the socio-economic aspects of the farmers involved in production because knowledge, skills and attitudes are very important in terms of effective application (Kasuga *et al*., 2003).

Before (pre-bloom) and during flowering (full bloom) phenological phases (Appendix 1), effective control can be obtained by spraying a mixture of insecticide for *Helopeltis* pest and a fungicide (Ohler, 1979; Ponte, 1984; Milheiro and Evaristo, 1994; Freire *et al*., 2002; Adejumo, 2005). A number of protective and systemic fungicides have been reported to be effective against cashew anthracnose on both seedlings as well as adult plants: dithianon, anilazin, benomyl, bitertanol, triadimenol, triforin, captafol, maneb, dimethoate, mancozeb, thiram, thiophanate, Bordeaux mixture, copper oxychlorate and others (Ohler, 1979; Ponte, 1984; Milheiro and Evaristo, 1994; Freire and Cardoso, 1995; Freire *et al*., 2002; Cardoso and Freire, 2003; Adejumo, 2005). Copper based fungicides are the most frequently referred

(Ohler, 1979; Intini, 1987; Freire and Cardoso, 1995; Cardoso *et al*., 1999; Ikisan, 2000; Freire and Cardoso, 2002; Freire *et al*., 2002; Oliveira, 2002; Cardoso and Freire, 2003). In general, the frequency and intervals between applications depend on the prevalence of favorable conditions (Ponte, 1984). The spraying program may involve one or two alternated molecules (Oliveira, 2002; Adejumo, 2005). On other crops other fungicides have been found efficient against *Colletotrichum* species (Waller, 1992).

Generally chemical control methods have raised global awareness of the adverse effect of chemical residues on human health and the environment (Waller *et al*., 1992; Smith *et al*., 1997). The effectiveness of chemicals in controlling *C. gloeosporioides*, is influenced by economics related to high cost of inputs at farmers' levels and manufacturing industries (Waller *et al*., 1992; De Jager, 1999), fungicides tolerance by the pathogen (Korsten *et al*., 1995, Dik *et al*., 1998), lack of effective regulations and their limited enforcement (De Jager, 1999) and sometimes inadequacy in terms of correct timing of application by farmers, and repetitive applications under rain conditions (Waller, 1992). Truly, spraying fungicides on cashew in Brazil was found to be ecological and economically risky and to some extent less practical on big trees (Cardoso *et al*., 1999). Thus the use of resistant cultivars is by far the best option in agricultural production systems (Waller, 1992).

In Mozambique, chemical control of cashew anthracnose was for the first time recommended by Dhindsa and Monjana (1984). Since then, only copper based fungicides have been used, basically for seedling protection in the nurseries (Lopes *et al*., 1983). During the crop season of 2003/2004 copper was used on a large scale for tree protection suggesting a greater awareness of the seriousness of the disease. However, the economic relevance in terms of return on investment of such applications is still unknown particularly when fungicides are also applied for cashew powdery mildew (*Oidium anacardii* Noack) control.

Regardless of the pathogens' penetration mechanism, different histochemical compatible and incompatible host reactions have been observed and accumulation of phenolic compounds is typical in cases of host resistance (Freire and Cardoso, 2003). Sources of partial resistance have been identified from Guine Bissau, Brazil, Nigeria and India (Milheiro and Evaristo, 1994; Muniz *et al*., 1998; Anon[a], 1999; Cardoso *et al*., 1999; Freire *et al*., 2002; Adejumo, 2005). However it should be noted that the goal of resistant cultivars in perennial crops is not

easy to attain because breeding and selecting requires a long term investment and resistance must be durable enough to cope with emergence of new strains of the pathogen (Waller, 1992).

Biological control can be obtained through the use of antagonistic micro-organisms, such as *Bacillus subtilis*, which inhibit the growth of *C. gloeosporioides* (Piteira, 1996). This was based on *in-vitro* interactions. When *B. subtilis* was tested against cashew powdery mildew (*Oidium anacardii* Noack) on inflorescences, the product showed no phytotoxicity to cashew and limited efficacy (Uaciquete, 2004) and later was found to be less competitive compared to classical fungicides (Mangue, 2001). The use of biocontrol in post harvest management of anthracnose has been well reported (Korsten *et al*., 1991; Korsten *et al*., 1995) and to some extent in field applications in avocado (Korsten *et al*., 1995) and mango (De Jager, 1999). There is no evidence of the use of this strategy on pre or post-harvest disease management in cashew.

Fortunately, resistance to *Colletotrichum* diseases has often been shown to be of polygenic and durable nature (Waller, 1992). Anthracnose management programs are based in general on identification of resistant germplasm, chemical control, biological and cultural control or any combination of these methods (Ohler, 1979; Hill and Waller, 1988; Freire *et al*., 2002; Lopez and Lucas, 2002).

2.3. Conclusions

Cashew is an important plant for both developing and developed countries. The value of the crop involves the full supply chain including production and processing and its significant impact on food security and job creation. However, its production in Mozambique has been characterized by crisis and low investment. The importance of the crop in the Mozambican economy and the potential provided by natural agro-ecological environment still justifies the need for further investments in good production techniques such as disease management.

Anthracnose is the second most important cashew disease in Mozambique. The causal agent (*C. gloeosporioides*) is within a morphological and genetically complex taxonomic group. A number of technical tools are available but only a few of them have been used on cashew patho-system. Previously generated knowledge on cashew-*Colletotrichum* patho-system has

not been directly applied onto disease management strategies. Therefore further research on cashew *Colletotrichum* diversity must focus on its association with adequate disease control approaches like in other tree crops. Host genotype and environment related symptoms variations have not been highlighted. Furthermore, the complexity caused by the simultaneous infection with other pathogens like *Pestalotia* sp. on the same tissue and resemblance with other diseases like the recently describe blight, (*Cryptosporiopsis* sp.) remains un-resolved. This suggests that the nature of symptoms observed in each case may require a clarification if proper disease diagnosis is to be made.

Most of the available information on cashew anthracnose epidemics was generated from Brazil, the country of origin of cashew. Little has been developed from other countries. However, many research findings are found on other tropical fruit crops. This fact may suggest that there have been no epidemics, probably due to environmental variations and host germplasm diversity restrictions. Alternatively, the epidemics have not been reported because human capacity and resources have been insignificant. However, environmental concerns associated with the use of fungicides and the increasing awareness on the value of integrated management approaches demands international research efforts. Similarly, the current increase on plantations area and importation of dwarf and highly productive cultivars have been triggering further needs for localized research on epidemiology of cashew anthracnose in Mozambique.

Cashew anthracnose is widely distributed in all cashew growing countries but information on its relative economic importance locally or over time is limited. This is despite the availability of numerous techniques for disease assessment. The phenomenon may be related to the fact that cashew is produced mainly in developing countries with little resources and few specialized personnel.

Management of *Colletotrichum gloeosporioides* complex can be achieved through a combination of various methods. Resistance and chemical control appear to be the most successful so far. The use of scientifically proven cashew tolerant cultivars in Mozambique has not been attained. Although, fungicides may be adopted based on international recommendations, doses, application rates, intervals and the economics of such programs still require refinements as per the local socio-economic and environmental conditions.

References

Adejumo, T. O. 2005. Crop protection strategies for major diseases of Cocoa and Cashew in Nigeria. *African Journal of Biotechnology 4: 143-150*.

Agrios, G.N. 2005. Plant Pathology. Fifth Edition. Elsevier Inc..952 p.

Alexopoulos, C.J. & Mims, C.W. 1979. Introductory Mycology. Third Edition. John Wiley & Sons, Inc. 632 p.

Amorati, R., Attanasi, O.A., Favi, G., Menichetti, S., Pedulli, G.F., & Viglianisi, C. 2011. Amphiphilic antioxidants from cashew nut shell liquid (CNSL) waste. *Organic and Biomolecular Chemistry 9:1352-1355*.

Anon, 1997. Decreto no 43/97, de 23 de Dezembro. Boletim da Republica I Serie, Numero 52. Governo de Mocambique. Maputo. 6 p.

Anon, 1998. Componente Producao. Plano Director do Caju. Instituto de Fomento do Caju INCAJU. Maputo. 68 p.

Anon.[a], 1999. Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew (*Anacardium occidentale* L.). Pages 112-116 *in*: Summary reports of European commission supported STD-3 projects (1992-1995). CTA. [online http://intership.cta.int/pubs/std/vol2/pdf/221/pdf] 111-116, March 25, 2009.

Anon.[b], 1999. Sector do Caju. Resumo da estrategia de produção do caju e analise de algumas medidas de politica. Incaju, Ministério da Agricultura, Maputo. 73 p.

Anon., 2006. Balanço do primeiro plano director do caju. Ministério da Agricultura e desenvolvimento rural. Instituto de Fomento do caju (INCAJU). Maputo. 125 p.

Anon., 2007. Reuniao Anual da African Cashew Alliance (ACA). Subsector do Caju em Mocambique: Evolucao e Perspectivas. Http/www/africancashewalliance.com. March 14, 2008.

Anon., 1992. Cashew Cultivation Handbook. Oltremare, Bologna. 53 p.

Aksoy, M.A. & Yagci, F. 2012. Mozambique cashew reform revisted. Policy research working paper 5939. The world bank poverty reduction and economic network. International trade department. 38 p.

Arauz, L.F. 2000. Mango Anthracnose: Economic and impact and current options for integrated management. *Plant Disease 84: 600-611*.

Archak, S., Gaikwad, A.B., Gautam, D., Rao, E.V.V.B., Swamy, K.R.M. & Karihaloo, J.L. 2003. DNA fingerprinting of Indian cashew (*Anacardium occidentale* L.) varieties using RAPD and ISSR techniques. *Euphytica 230: 397-404*.

Ascenso, J.C. & Dunan, I.E. 1998. Cashew processing and marketing. Pages 189-194 *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. &

Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Asolkar, T., Desai, A.R. & Singh, N.P. 2011. Molecular analysis of cashew genotypes and their half-sib progeny using RAPD marker. *Research Article, Biotecnol, Bioinf. Bioeng. 1: 495-504*.

Augustin, A. 2001. Utilization of cashew apple. Pages 57-66 in: Cashew the Millennium Nut. World Cashew Congress, 23-25 February. Souvenir. International Convention Centre, Kochi.

Bailey, J.A., O'Connell, R.J., Pring, R.J. & Nash, C. 1992. Infection strategies of *Colletotrichum* species. Pages 88-120 *in*: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Baker, R.E.D., Crowdy, S.H. & Mckee, R.K. 1940. A review of latent infections caused by *Colletotrichum gloeosporioides* and allied fungi. *Tropical Agriculture 17: 128-132*.

Barros, L.M.; Paiva, J.R. & Cavalcante, J.J.V. 2001. Cashew (*Anacardium occidentale* L.): A review of Brazilian current research situation. Pages 47-51, *in*: Cashew, the Millennium Nut. World Cashew Congress 23-25 February. International Convention Centre. Kochi.

Barros, L. M., Paiva, J.R., Crisótomo, J. R. & Cavalcante, J.J.V. 2002. Botânica, origem e distribuição geografica. Pages 18-20 *in*: Barros (ed.). Produção, produção e aspectos técnicos. Embrapa. Brazil.

Behrens, R. 1996. Cashew as an Agro-forestry Crop. Prospects and Potentials. Tropical Agriculture 9. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) Gmbh, Eschborn. 83 p.

Bisanda, E.T.N. 1998. Cashew nut shell liquid (CNSL) resins as matrix in plant fibre reinforced composites. Pages 217-221, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Cardoso, J.E., Cavalcante, J.J.V., Cavalcante, M. De J.B., Aragao, M. Do L. & Felipe, E.M. 1999. Genetic Resistance of dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mould and angular leaf spot. *Crop Protection 18: 23-27.*

Carvalho, T. & Mendes, O. 1958. Doencas de Plantas em Mocambique. Reparticao de Sanidade Vegetal. Minerva Central. Lourenco Marques. 84 p.

Casulli, F. 1975. L'antracnosi dell'anacardio in Tanzania. *Revista di Agricoltura Subtropicale e Tropicale 73:451-456.*

Chabi Sika, K., Adoukonou-Sigbadja, H., Ahoton, L.E., Adebo, I., Adigoun, F.A., Saidou, A., Kotchoni, S.O., Ahanchede, A. & Baba-Moussa, L. 2013. Indigenous knowledge and traditional management of cashew (*Anacardium occidentale* L.) genetic resources in Benin. *Journal of Experimental Biology and Agricultural Sciences 1:375-382*.

Cumbi, S. 1998. Cashew production in Mozambique. Pages 14 and 15 *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H.; Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd, Reading.

Da Matta, E.A.F. & Lellis, W.T. 1973. Fungicidas e adubacao no controle da "Queima do Cajueiro". *Boletim do Instituto Biologico de Bahia 12: 37-40*.

Daramola, B. 2013. Assessment of some aspects of phytonutrients of cashew Apple juice of domestic origin in Nigeria. *African Journal of food science 7: 107-112*.

De Araujo, J. & Da Silva, V.V. 1995. Cajucultura. Modernas Tecnicas de Produção. Ministerio da Agricultura, do Abastecimento e da Reforma Agraria. Empresa Brasileira de Pesquisa Agropecuaria - EMBRAPA. Centro Nacional de Agroindustria tropical-CNTP, Fortaleza. 298 p.

De Jager, E.S. 1999. Microbial ecology of the mango flower, fruit and leaf surfaces. M. Sc. (Agric) thesis. University of Pretoria, Pretoria.

Dhindsa, P.P. & Monjana, A.M. 1984. Index of plant diseases and associated organisms of Mozambique. *Tropical Pest Management 30: 407-429*.

Dik, J., Verhaar, M.A. & Belanger, R.R. 1998. Comparison of three biological control agents against cucumber powdery mildew (*Sphaerotheca fuliginia*) in semi-commercial scale glasshouse. *European Journal of Plant Pathology 104: 413-423.*

Dodd, J.C., Estrada, A. & Jeger, M.J. 1992. Epidemiology of *Colletotrichum gloeosporioides* in the tropics. Pages 309-325 in: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Dodd, J.C., Estrada, A., Matcham, J., Jeffries, P. & Jeger, M.J. 1991. The effect of climatic factors on *Colletotrichum gloeosporioides*, causal agent of mango anthracnose in the Philippines. *Plant Pathology 40: 1-8*.

Duke, A.J. 1989. Handbook of Nuts. CRC Press Inc., Florida. 343 p.

Estrada, A.B., Dodd, J.C. & Jeffries, P. 2000. Effect of humidity and temperature o conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. *Plant Pathology 49: 608-618*.

FAOSTAT. 2013. http://faostat.fao.org/site/399/dfault.aspex. December, 2013.

Ferrão, J.E.M. 1995. O cajueiro (*Anacardium occidentale* L.). Ministerio do planeamento e da administracao do territorio. Secretaria de Estado da Ciencia e Tecnologia. Instituto de Investigação Cientifica Tropical. Lisboa. 298 p.

Ferrão, J.E.M. 1999. Fruticultura Tropical: Especies com frutos comestiveis -Volume I. Instituto de Investigacao Cientifica Tropical; Missao de Macau. Lisboa. 621 p.

Filgueiras, H. A. C. & Alves, R.E. 2001. Cashew apple for fresh consumption in Brazil. Pages 53-55 *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Freire F. C. O. & Cardoso, J. E. 1995. Doencas do cajueiro Pages 250-267. *In*: Cajucultura. Modernas tecnicas de producao. De Araujo, J. P. P. & Da Silva, V. V. (eds). EMBRAPA-CNPAT. Fortaleza.

Freire, F.C.O., Cardoso, J.E., Dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21: 489-494*

Freire, F.C.O. & Cardoso, J.E. 2003. Doencas do cajueiro. Pages 192-225 in: Doencas de Fruteiras tropicais de interesse agroindustrial. Freire, F.C.O., Cardoso, J.E., Viana, F.M.P. (eds.). Embrapa Informacao Tecnica. Brasilia.

Gonzaléz, E. & Sutton, T. B. 2004. Population diversity within isolates of *Colletotrichum* spp. Causing *Glomerella* Leaf Spot and Bitter Rot of Apples in Three Orchards in North Carolina. *Plant Disease 88: 1335-1340*.

Gordon, A., Friedrich, M., da Matta, V.M., Moura, C.F.H. & Marx, F. 2012. Changes in phenolic composition, ascorbic acid and antioxidant capacity of cashew apple (*Anacardium occidentale* L.) during ripening. *Fruits 67: 267-279*.

Gunjate, R.T. & Patwardhan, M.V. 1995. Cashew. Pages 509-521 *in*: Handbook of Fruit Science and Technology. Production, Composition, Storage and Processing. Salunke, D.K. & Kadam, S.S. (Eds). Marcel Dekker Inc.. New York.

Guyot, J., Omanda, E.N. & Pinard, F. 2005. Some epidemiological investigations on *Colletotrichum* leaf disease on rubber tree. *Crop Protection 24: 65-77*.

Hartman, H.T., Flocker, W. J. & Kofranek, A.A. 1981. Cashew (*Anacardium occidentale L.*) Anacardiaceae. Plant Science. Growth, Development and Utilization of Cultivated Plants. Prentice-Hall Inc., New Jersey. 676 p.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C. & Pegler, D.N. 1995. Ainsworth & Bisbys Dictionary of the Fungi. Eighth edition. International Mycological Institute. CAB International. 616 p.

Hill, D.S. & Waller, J.M. 1988. Pests and diseases of tropical crops. Field handbook, low priced edition. Longman. London. 432 p.

Ikisan, 2000. Cashew. Crop Information. http://www.Ikisan.com. January 27, 2008.

INE, 2011. Censo Agro-Pecuario 2008-2010. Resultados definitivos. The National Institute for Statistics. Maputo.

Intini, M. 1987. Phytopathological aspects of Cashew (*Anacardium occidentale* L.) in Tanzania. *International Journal of Tropical Plant Diseases 5: 115-130*.

Ivey, M.L.L., Nava-Diaz, C. & Miller, S.A. 2004. Identification and Management of *Colletotrichum acutatum* on immature Bell Peppers. *Plant Disease 88: 1198-1204.*

Jeffries, P., Dodd, J.C., Jeger, M. J. & Plumbley, R.A. 1990. The biology and control of *Colletotrichum* species on tropical fruit crops. *Plant Pathology 39: 343-366*.

Kanji, N., Vijfhuizen, C., Artur, L. & Braga, C. 2004. Liberalisation, gender, and livelihoods: The Mozambique cashew nut case. Summary report. International Institute for Environment and Development. Nederlanden. 35 p.

Kasuga, L.J., De Waal, D., Boma, F. & Topper, C.P. 2003. The approach and process of the Integrated Cashew Management Programme. Pages 98-118 *in*: Topper, C.P. & Kasuga, L.J. (eds). Knowledge transfer for sustainable tree crops development. A case history of the Tanzanian Integrated Cashew Management Programme. Biohybrids Agrisystems Ltd, Reading.

Korsten, L., Van Harmelen, M.W.S., Heitmann, A., De Villiers, E. & De Jager, E. 1991. Biological control of post-harvest mango diseases. *South African Mango Growers Association Yearbook 11: 65-67.*

Korsten, L., De Jager, E.S., De Villiers, E.E., Kotzé, J.M. & Wehner, F.C. 1995. Evaluation of epiphytes isolated from avocado leaf and fruit surfaces for biocontrol of avocado postharvest diseases. *Plant Disease 79: 1149-1156*.

Korsten, L., Wehner, F.C., De Villiers, E.E. & Kotzé, J.M. 1997. Field sprays of *Bacillus subtilis* and fungicides for control of preharvest fruit diseases of avocado in South Africa. *Plant Disease 81: 455-459.*

Kubo, Y., Furusawa, I., Ishida, N. & Yamomoto, M. 1992. Relation of appressorium pigmentation and penetration of nitrocellulose membranes by *Colletotrichum lagenarium. Phytopathology 72: 498-501.*

Kumar, R. 1995. Matieres Premiers. L'industrie de la noix de cajou profite-t-elle `a l'Afrique? *March'es Tropicaux 2581: 897-899*.

Lakshmi, B.K.M., Reddy, P.N. & Prasad, R.D. 2011. Cross-infection potential of *Colletotrichum gloeosporioides* Penz. Isolates causing anthracnose in subtropical fruit crops. *Tropical Agriculture Research 22:183-193*.

Leite, J.P. 1999. A guerra do caju e as relacoes Mocambique-India na epoca pos-colonial. Documentos de traballho no. 57, CesA. Lisboa.

Lihong, L., Zetan, C., Chenghai, C. & Congfa, L. 2005. Curso de Formação China-Moçambique sobre o cultivo do cajueiro. Tecnologia do Cultivo do Cajueiro. Ministerio da Agricultura da Republica Popular da China. Beijing. 73 p.

Lim, T. K. & Singh, G. 1985. Disease and Pest Problems of Cashew in Malaysia. Pages 139-144, *in*: Rao, E.V.V.B & Khan, H.H. (eds.). International Cashew Symposium. Research and Development, 12-15 March, 1979, Cochin. Technical Communications of International Society for Horticultural Science. *Acta Horticulturae (ISHS) 108:139-144.*

Lopes, J.G., Vicente, J. & Petinga, M.R.J. 1993. Agrotecnia do Caju. Ministerio da Agricultura. Serviços Nacionais de Extensão Agraria.Serie Culturas. Maputo. 46 p.

Lopez, A.M.Q. & Lucas, J.A. 2002. Effects of plant defense activators on anthracnose disease of cashew. *European Journal of Plant Pathology 108: 409-420*.

Lopez, A.M.Q. and Lucas, J.L. 2010. *Colletotrichum* isolates related to anthracnose of cashew trees in Brazil: Morphological and Molecular description using LSU and rDNA sequences. *Braz. Arch. Biol.Tecnol. 53:741-752*.

Manners, J.M., Masel, A., Braithwats & Irwin, J.A.G. 1992. Molecular analysis of *Colletotrichum gloeosporioides* pathogenic on the tropical pasture legume *Stylosanthes*. Pages 251-268, *in*: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Mangue, J.R. 2001. Controlo Quimico e Biologico de Oidio do Cajueiro (*Oidium anacardii* Noach). Tese de Licenciatura em Agronomia. Faculdade de Agronomia e Engenharia Florestal. Universidade Eduardo Mondlane. Maputo.

Mazzetto, S.E., Oliveira, L.D.M., Lomanaco, D. & Veloso, P.A. 2012. Antiwear and antioxidant studies of cardanol phosphate ester additives. *Brazilian Journal of Chemical Engineering 29:519-524*.

Mbulanya, G.G.N. 1998. The wear characteristics of frictional elements based on cashew nut shell liquid. Pages 206-210, *in*: Topper, C.P.; Caligari, P.D.S.; Kullaya, A.K.; Shomari, S.H.; Kasuga, L.J.; Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd. Reading.

Menge, D., Makobe, M., Shomari, S. and Tiedemann, A.V. 2013. Development and validation of a diagrammatic scale for estimation of cashew blight for epidemiological studies. *International Journal of Advanced Research 1:26-38*.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do cajueiro. Cultivar. Associação de Tecnicos de Culturas Tropicais, Porto. 204 p.

Mills, P.R., Hadson, A. & Brown, A.E. 1992. Molecular differentiation of *Colletotrichum gloeosporioides* isolates infecting tropical fruits. Pages 269-287 *in*: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Mitchell, J.D. & Mori, S.A. 1987. The cashew and its relatives (*Anacardium: Anacardiaceae*). The New York Botanical Garden. New York. 76 p.

Mosha, E., Kikoka, L., Masawe, W. & Kimomwe, H. 1998. Rural savings and credit cooperatives (saccos): Potential Rural Financial Institutions for Agricultural Development in Tanzania. Pages 222-227, *in*: Topper, C.P.; Caligari, P.D.S.; Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International cashew and Coconut conference, 17-21 February, 1997. Biohybrids International Ltd, Reading.

Muniz, M.F.S., Lemos, E.E.P., Varzea, V.M.P., Rodrigues, C.J. Jr & Bessa, A.M.S. 1998. Characterization of *Colletotrichum gloeosporioides* (Penz.) Sacc. Isolates and resistance of cashew (*Anacardium occidentale* L.) to the pathogen. Pages 249-253, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference, 17-21 February, 1997. Biohybrids International Ltd, Reading.

Nair, M.K., Bhaskara, R.E.V.V., Nambiar, K.K.N. & Nambiar, M.C. 1979. Cashew (*Anacardium occidentale* L.). Monograph on Plantation Crops 1. Central Plantation Crops Research Institute, Kerela. 169 p.

Nair, S. 2001. Indian Cashew Industry. Pages 87-92, *in*: Cashew, the Millennium Nut. World Cashew Congress 23-25 February. Proceedings. International Convention Centre. Kochi.

Nambiar, K.K.N. 1978. Controlling Cashew Diseases. *Indian Farming 28: 17-18.*

Nathaniel, A. K., Mfilinge, A. G., Lamboll, R. & Topper, C.P. 2003. Socio-economic studies undertaken in relation to cashew growing households. Pages 61-84 *in*: Topper, C.P. & Kasuga, L.J. (eds). Knowledge transfer for sustainable tree crops development. A case history of the Tanzanian Integrated Cashew Management Programme. Biohybrids Agrisystems Ltd.. Reading.

Nayar, K.G. 1998. Cashew: A versatile nut for health. Pages 195-200 *in*: Topper, C.P.; Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference 17-21 February, 1997. Biohybrids International Ltd. Reading.

O'Connell, R.J., Nash, C. & Bailey, J.A. 1992. Lectin Cytochemistry: A new approach to understanding cell differentiation, pathogenesis and taxonomy in *Colletotrichum*. Pages 66-87 *in*: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Ohler, J.G. 1979. Cashew. Communication no. 71. Department of Agricultural Research of the Royal Tropical Institute, Amsterdam. 260 p.

Oliveira, V.H. 2002. Sistemas de Producao 1. Cultivo do Cajueiro Anao Precoce. Embrapa-Agroindustria Tropical. Fortaleza. 40 p.

Pell, S.K. 2004. Molecular systematics of the cashew family (Anacardeaceae). Ph.D. thesis. Louisiana State University. 144 p.

Pillai, P.G. 2001. Cashew nuts. Nutritious snacks that naturally taste great. Pages 73-77, *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Pakela, Y.P. 2003. Interaction between *Colletotrichum dematium* and cowpea. Ph. D. thesis. University of Pretoria.

Piteira, M.C.C. & Rodrigues, Jr. C. J. 1999. *Colletotrichum gloeosporioides* Penz. of cashew (*Anacardium occidentale* L.). Morphocultural studies and pathogenicity tests using cross inoculations on other tropical fruits. Estação Agronómica Nacional, Oeiras (Portugal). Minutes of the 2nd Biennial Meeting of the Portuguese Phytopathology Society. P. 288.

Ponte, J.J. 1984. Doencas do cajueiro no Nordeste Brasileiro. Documento 10. EMBRAPA-Departamento de Difusao de Tecnologia. Brasilia. 51 p.

Rao, E.V.V.B. 1998. Cashew Crop Improvement Programmes in India. Pages 141-145, *in*: Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J.; Masawe, P.A.L. & Mpunami, A.A. (eds). Proceedings of the International Cashew and Coconut conference 17-21, February, 1997. Biohybrids International Ltd, Reading.

Rickson, R.F. & Rickson, M.M. 1998. The cashew nut, *Anacardium occidentale* (Anacardiaceae), and its perennial association with ants: extrafloral nectary location and the potential for ant defense. *American Journal of Botany 85: 835-849*.

Salam, M. A. 2001. Cashew research and development, Indian scenario, Pages 31-41 *in*: Cashew the millennium nut. World cashew congress, 23-25 February. Souvenir. International Convention Centre. Kochi.

Samal, S., Rout, G.R. & Lenka, P.C. 2003. Analyis of genetic relationships between populations of cashew (*Anacardium occidentale* L.) by using morphological characterization and RAPD markers. *Plant Soil Environ., 49:176-182.*

Simon, C.R. 2001. Cashew nut as a constituent of healthy diet. Pages 67-72 *in*: Cashew, the millennium nut. World Cashew Congress 23-25 February. Souvenir. International Convention Centre. Kochi.

Silinto, B.F. 2005. Influencia de alguns factores ambientais no desenvolvimento da antracnose nas folhas do cajueiro (*Anacardium occidentale* L.). Trabalho de Licenciatura. Departamento de Ciencias, Universidade Eduardo Mondlane. Maputo.

Silva, A.F. 1961. O Cajueiro. Edicao do Gazeta do Agricultor. Serie B Divulgacao no. 20. Lourenco Marques. 26 p.

Swart, G.M. 1999. Comparative study of Colletotrichum gloeosporioides from avocado and mango. Ph. D. thesis. Microbiology. University of Pretoria.

Sundararaju, D. & Babu, PC.S. 1999. Recent research on management of neem mosquito bug on cashew. *Cashew 13:51-53*.

Sutton, B.C. 1966. Development of fructifications in *Colletotrichum graminicola* (Ces.) Wilson and related species. *Canadian Journal of Botany 44: 887-897*.

Tandjiekpon, A. 2005. Caracterisation du system agroforestier a base d'anacardier (Anacardium occidentale Linnaeus) en zone de savane au Benin. Diplomed' Etudes Approfundies (DEA), FLASH/UAC:104p.

Topper, C.P. & Caligari, D.S.P. 2003. Challenges for tree crops, research and extension in rural development. Pages 1-42, *in*: Topper, C.P. & Kasuga, L.J. (eds). Knowledge transfer for sustainable tree crops development. A case history of the Tanzanian Integrated Cashew Management Programme. Biohybrids Agrisystems Ltd, Reading.

Uaciquete, A. 2004. Epidemiology and control of powdery mildew (*Oidium anacardii* Noack) on cashew (*Anacardium occidentale* L.) in Mozambique. M. Sc. (Plant Pathology) thesis. University of Pretoria, Pretoria.

Vivek, M.N., Manasa, M., Pallavi, S., Sachidananda Swamy, H.C. & Prashith Kekuda, T.R. 2013. Antibacterial potential of cashew apple (*Anacardium occidentale* L.) juice against clinical isolates of *Staphylacoccus aureaus* and *Staphylacoccus mutans*. Short communication. *Science, Technology and Arts Research Journal 2:144-146*.

Walker, T., Pitoro, R., Tomo, A., Sitoe, I., Salencia, C., Mahanzule, R., Donavan, C. & Mazuze, F. 2006. Estabelecimento de prioridades para a investigacao agraria no sector publico em Mocambique baseado nos dados do trabalho de inquerito agricola (TIA). Instituto de Investigacao Agraria de Mocambique. Maputo.

Waller, J.M. 1992. *Colletotrichum* Diseases of Perennial and other Cash Crops. Pages 166-185 *in*: Bailey, J.A. & Jeger, M.J. (eds). *Colletotrichum*: Biology, Pathology and Control. British Society for Plant Pathology.

Wild, M. 1994. Various Subtropical Crops. Institute for Tropical and Subtropical Crops, Nelspruit.

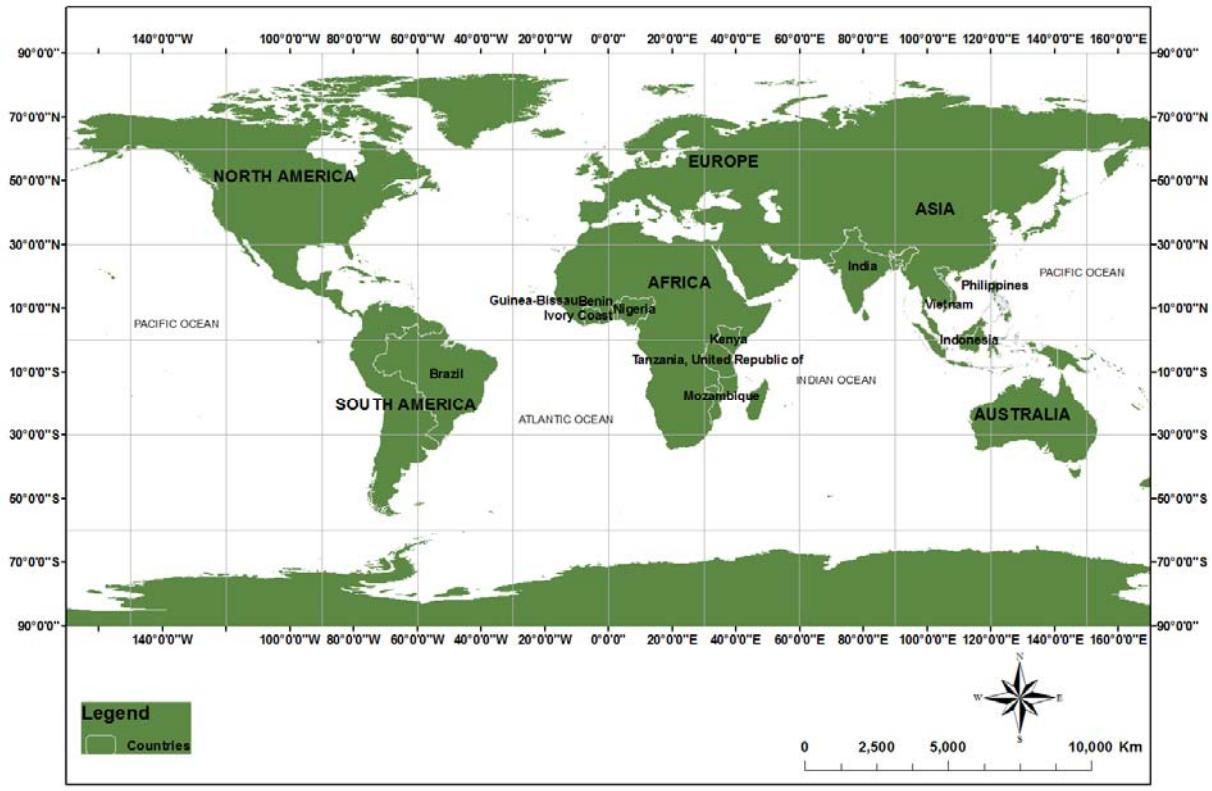


Figure 2.1: Worldwide main cashew producing countries. Modified from Araujo & Silva, 1995.

Table 2.1: Impact projection of the cashew sector revival in Mozambique, between 2005-2010.

Items/crop season	2005/6	2006/7	2007/8	2008/9	2009/10
Processing capacity(tones)	22700	36500	50000	61140	74200
Forex income ($1000)	21034	35542	49713	61146	73773
Processing units	25	29	33	37	43
Work posts	6356	10220	14000	17220	20776

Source: Carlos Costa, 2006 (*personal communication*).

Impacts		First production peak 216 000 Tones		Production crisis, 18 000 Tones			Industrial crisis, 10000 job lost	Second production peak, 104 000 Tones	Third production peak	Production decline
Year	**1940/50**	**1972/73**	**1975**	**1982/83**	**1994/5**	**1997**	**1999/2000**	**2004/5**	**2010/11**	**2012**
Events	Planting cashew trees		National Independence		Privatization & liberalization of cashew industry	Creation of the cashew promotion institute		Policies and climate in favour of cashew production		International price decline

Figure 2.2: Socio-political events over time and their impact on cashew nut production in Mozamique. Modified from Anon., 2007.

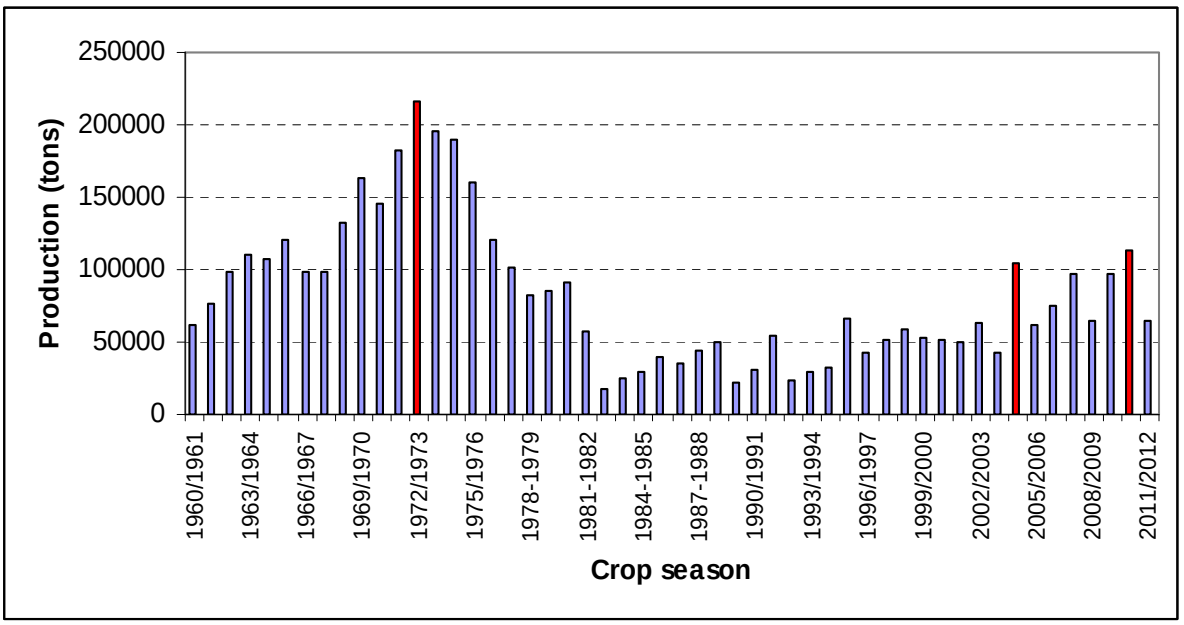


Figure 2.3: Cashew raw nuts production in Mozambique from 1960 to 2012, showing the maximum (1972/3) and minimum (1982/3) ever reached. Source: National Cashew Promotion Institute, 2013 (*personal communication*).

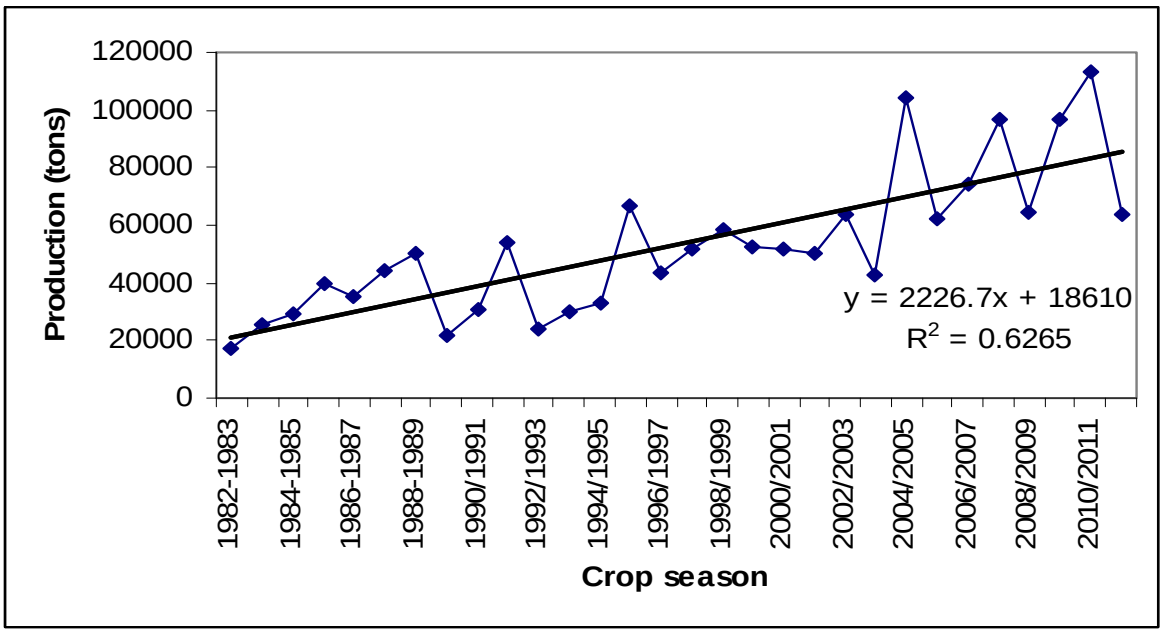


Figure.2.4: Growth trend of Cashew raw nuts production in Mozambique from 1983 to 2006, showing a maximum (2004/2005) and minimum (1989/1990) over the previous 20 years period. Source: National Cashew Promotion Institute, 2013 (*personal communication*).

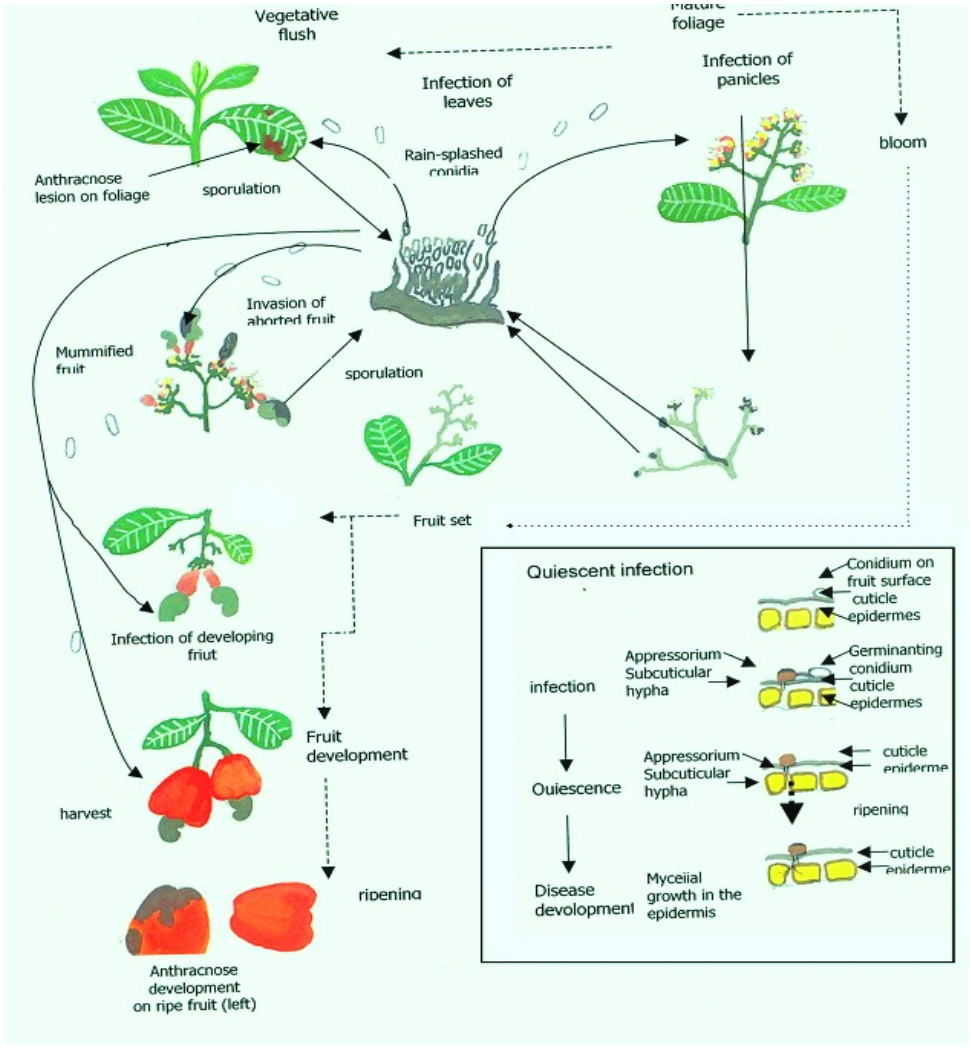


Figure 2.5: Anthracnose disease cycle on cashew. Modified from Arauz, F. L. (2000).

Table 2.2: Summary of cashew anthracnose dispersion over the years and countries and the authors of the referred articles

Reference	Baker *et al.*, 1940	Carvalho & Mendes, 1958	Casulli, 1975	Nambiar, 1978	Da Matta & Lellis, 1973	Lim & Singh, 1979	Intini, 1987	Muniz *et al.*, 1998
Year	**1940**	**1958**	**1960**	**1965**	**1973**	**1979**	**1987**	**1998**
Country	Trinidad Tobago	Mozambique	Tanzania	India	Brazil	Malaysia	Others	Guiney Bissau

CHAPTER THREE: PREVALENCE AND MANAGEMENT OF ANTHRACNOSE (*COLLETOTRICHUM GLOEOSPORIOIDES* PENZ.) ON CASHEW NURSERIES IN MOZAMBIQUE

3.1. Abstract

Cashew is an agricultural important crop in most developing countries providing an important nutrient source and addresses food needs and job creation throughout the crop value chain. A survey was conducted in 32 cashew nurseries throughout the cashew agro-ecological zones in Mozambique. The objectives of the survey were to identify the pathogen associated with reported blackening symptoms, assess its damage, identify weaknesses in management and formulate appropriate recommendations. For the survey the following approaches were adopted: pre-prepared questionnaire, incidence scoring on seedlings, sampling for laboratory isolation and identification of the causal agent. The anthracnose causal agent was sometimes isolated in association with other pathogens. The disease spread throughout the cashew nurseries with a higher incidence on rootstock (6.22%) than on grafted cashew seedlings (1.57%). Humid zones and seasons were more prone to disease epidemics with mean incidence as high as 7.60%. The disease was found to be prevalent between January and April. However, in specific agro-ecological zone, the disease was found throughout the year. The overall anthracnose incidence was less than 1%. The spread of the disease was associated with non-hygienic practices in the nurseries as well as the disease inductive irrigation methods in use. Adequate chemical treatment, avoidance of water splashes, disinfection of tools and scions, removal of sources of contaminations, etc., were recommended to improve the practices in each nursery.[1]

[1] Abstract published in *Proceedings of the National Agriculture Conference, Maputo (2011). Portuguese version in Appendix 6.*

3.2. Introduction

Plant mortality rate for cashew in Mozambique is estimated at about 1 000 000 trees per year (Anon., 2001). This fact, combined with the continuous aging of the trees (Milheiro and Evaristo, 1994), has justified the need for establishment of new plantations. But, cashew seedling production has been historically dominated by the public sector (Anon., 1998). Private organizations, non-governmental organizations (NGOs) and rural communities have created temporary nurseries throughout the country.

Until 1998, the national seedling production capacity was estimated at 700 000 units per year (Anon., 1998). In response to high rate of mortality, the production capacity was expanded to 1 500 000 units annually (Anon., 2007) which is less than the aim of producing 1 850 000 seedlings annually as approved in the master plan for five years till 2006 (Anon., 1998). Today, data from the National Institute for Cashew Development (INCAJU) points at a total of 255 nurseries of which 31 are public, 196 are community owned and 26 belong to private organizations.

The above quantitative shift from 700 000 to 1 850 000 seedlings on annual targets has triggered a serious of problems such as the recurrent occurrence of pests and diseases in the nurseries, shortage of water for irrigation and insufficiency of transport for distribution of seedlings to the widely scatted farmers. Additional limitation is the time pressure: under the current grafting technology, quantitative targets must be achieved in five months, from November to March (Anon., 1998). But, in this period, scions are not available, the rate of grafting success is lower, there is no rainfall for transplantation and more importantly, experienced workers have been reluctant to shift from softwood to other techniques such as budding.

Diseases and pests reported from nurseries included powdery mildew, anthracnose, mosquito bug (*Helopeltis* sp.) and leaf miner (*Phyllocnists* sp.) (Lopes, 1994). But until recently, pest and disease damage on the nurseries was negligible. Consequently, nursery management strategies were not part of the 1998 INCAJUs´ master plan for cashew production. Nevertheless, a high seedling mortality rate estimated at 45% over a period of three years was observed (Anon., 2005). This survey was conducted in order to: (i) Identify causes of seedling mortality associated with observed leaf blackening symptoms, (ii) assess the extent of

damage, (iii) identify weaknesses in management and (iv) formulate appropriate management recommendations.

3.3. Material and Methods

3.3.1. General procedures and disease prevalence

The survey was conducted in 32 representative nurseries throughout the main cashew producing regions of Mozambique during the period from April to May, 2007. A two part questionnaire was used (Appendix 11). The first part aimed at the workers in charge of the nurseries and the second part targeted those involved in preparation and collection of scions from the mother trees.

Both categories of informants were interviewed using the questionnaires with included photos of anthracnose symptoms on seedlings or shoots for easy identification. A photo of similar disease symptoms was also included to facilitate disease recognition and thus making the communication easier and to determine for possible confusion with other diseases.

Other relevant information was noted during the survey, either in the form of additional notes, or as photographic records using a digital camera. Geographic position of the nurseries was also recorded using a Garmin GPS, Map 76S Atl.v (Series number, 93702677, Garmin, Olathe, KS, USA). For some locations, the coordinates were downloaded through the computer software Google Earth, European technology, 2007.

3.3.2. Key disease related processes

The role of seed and soil or other seedlings as source of inoculum was examined in each nursery. The risk that the presence of infected cashew trees in the vicinity of a nursery (up to 300 meters) can pose was explored either by physical verification or through the questionnaire. Furthermore, the potential of scions as carriers of inoculum was also examined through the questionnaire. This included a critical analysis through the steps involved in the process of scion collection, transportation, conservation and grafting. In all steps involved, interviewees responsible for scions collection as well as those in charge of the nurseries were

requested to provide information on precautionary measures and disease management approaches taken to prevent transmission.

3.3.3. Spatial and temporal disease distribution in a nursery

The role of shedding (type and coverage), watering frequency and method were evaluated through the questionnaire and physical observations. Similarly, the possible role of the microclimate associated with either growth stage (age of the seedlings) or seedling density was also explored.

Arising from observations during the survey, further experiments on the effect of seedling density and age on disease development were conducted (CHAPTER V). Notes on other factors that may facilitate disease spread in the nursery were also taken into consideration. These included handling of the seedlings, transmission routes and the movement of workers. Finally respondents were requested to provide information on temporal distribution of the disease.

3.3.4. Analysis of data

Quantitative and qualitative data were all tabulated in spreadsheet (Microsoft Excel). Disease incidence was computed for each nursery and province, totals and means were tabulated. Geographic patterns of the disease prevalence were examined over the provinces, geographic position (GPS data) or agro-ecological zones (Anon., 1996) by overlapping maps of the same scale. Statistical frequencies of particular answers, percentages, totals and or averages and respective standard deviations were computed accordingly.

Data on temporal distribution of the disease were analyzed by highlighting in excel sheet the location and respective period (months) of occurrence. Periods of high disease incidence were noted by high frequency of locations with infected seedlings. Similarly, locations of continuous occurrence of the disease were noted over the time.

3.4. Results

3.4.1. Disease prevalence

Most interviewees (91.4%) responded to have noticed anthracnose symptoms from some of the mother trees during preparation and collection of scions. In one extreme scenario at Macia district, there was a reference that it would be hard to find a single tree that is completely free from anthracnose symptoms. The workers involved in scion preparation associated the disease with poor quality of scions (74.2%), meaning reduction of success at grafting (52.4%) or transmitting the disease on grafted seedlings (19.0%) and others did not know the effect (28.6%). Workers at the nursery believe that anthracnose does cause damage to seedlings (82.0%). Among these, 32.0% admitted that defoliation is the only damage observed while other 32% reported that severe infection could cause mortality of seedlings. Other respondents (36%) believe that the disease disappears upon grafting or because they spray the seedlings immediately after grafting and therefore never experienced a severe disease damage.

Out of the 32 nurseries surveyed, a total of 552 679 seedlings were assessed. Among these, 175 390 were rootstock and 377 289 grafted (Table 3.1). The cumulative anthracnose incidence for Zambezia province was as high as 28.29%. This was about six times the disease incidence recorded from other provinces (Table 3.1). Anthracnose incidence means on rootstock as well as grafted seedlings per province, district and location surveyed are presented in Table 3.2. The overall mean incidence on un-grafted seedlings was higher (6.22%) than that of grafted ones (1.57%) (Table 3.2).

In three cases of severe infection, leading to seedling mortality, the percentage of died was as follows: 30%, at Maganja da Costa District, 60% at Gile district and 15% at Meconta, recorded in seedling populations of 200, 1200 and 250 respectively.

3.4.2. Key disease related processes and precautionary measures

3.4.2.1. Soil

In some nurseries, e.g. at Nassuruma (Meconta district), the soil for seedlings is collected from under cashew trees canopies, up to 20 cm deep, after removal of superficial layer of

dried fallen leaves. Because some of the trees have symptoms of anthracnose and infected leaf debris are occasionally passed onto seedling soil, there is a potential for transmission of viable saprophytic spores.

3.4.2.2. Seeds

In particular nurseries in Inhambane and Gaza provinces, seeds are deepen in to a solution of fungicide before sowing, but in most cases they are not. In one case we noted that seeded pots, were being sprayed with fungicide (copper oxychloride) even before seed germination (Plate 3.1D). Very young seedlings, up to leaf five in general, had no symptoms of anthracnose. At more developed stages of the seedlings, when most leaves are severely infected, the first four, at the bottom, tend to show minor expansion of necrotic area. Because this observation has led to the hypothesis of age related seedling tolerance to anthracnose, a specific trial was established (CHAPTER V).

3.4.2.3. Infected adult plants and scion gardens

For most nurseries (84.4%) there was at least one anthracnose infected cashew tree at less than 300 meters distance. Such trees were sometimes used as shad for the seedlings within the nursery itself. At Nhacoongo nursery, were comparison was made, anthracnose incidence, under the tree canopy was 69.20% while under the 50% conventional polyethylene shedding, infected seedlings were less than 1%. Therefore, the role of these trees as source of inoculum was evident. In general, seedlings placed under the canopy or nearby (estimated 3 meters) were highly infected when compared to those located beyond.

Wounds by insects on tender shoots facilitate penetration of pathogens such as *Colletotrichum* sp. and *Phomopsis* sp. in the host plant (Ohler, 1979; Intini and Sijaona, 1983,). Mosquito bug and or its damage occurred in almost all nurseries despite its control with either cyhalotrin or cypermetrin. Elite clonal scions gardens were established close to the nurseries with a view to make them easily accessible. These clonal gardens are still young and include cashew dwarf types that produce new flushes throughout the year, a favorable condition for proliferation of mosquito bug (Sundararaju and Babu, 1999). This suggests that the recommended integrated pest and disease management at the nursery (Ohler, 1979; Intini and

Sijaona, 1983; Sundararaju and Babu, 1999; Freire *et al*., 2002,) must also aim at the nearby cashew scion gardens and other adult cashew trees to avoid recurrent infestation.

3.4.2.4. Scions

Scions are transported as small bunches, tied together as per the name of the mother tree either in dried weed or in banana stem husk. They are wetted and preserved under cool conditions in shed or buried (Plate 3.1F). No particular disease management measure is taken at this stage. But there is second stage of scion selection, if one escapes at mother tree level, is at grafting stage, when grafters make the grafting edges. ″Pencil scions″ (Plate 3.1G) detected are similarly rejected and the grafting knifes are immediately disinfected with either ethanol (70%) or lemon (Plate 3.1H). Four respondents, all from Gaza province, out of 31 countrywide do use fungicides for the treatment of scions before grafting. Like the seeds, before grafting, scions were deepened into a solution of fungicide and a grafting success increase of 20 to 30% was reported.

3.4.2.5. Mother trees

Precautionary measures taken by the workers in preparing the scions are based on exclusion of infected plant (45% of our respondents) or exclusion of infected scion shoot (45%). Infected plant or shoot is detected by the presence of characteristic necrotic symptoms on the leaves. But sometimes, because infected leaves may have fallen, scions will be rejected when the cutting edge has developed brown or black color (pencil scions).

The use of fungicides as preventive measure for anthracnose on mother trees is not common. Most of our respondents (75.8%) do not treat the mother trees chemically to ensure disease free scions. They believe (19.4%) that the presence of the symptoms has no effect on the rate of success at grafting as long as they can avoid the ″pencil scions″ and select disease free mother plants.

3.4.2.6. Infected seedlings

Handling of seedlings at the nursery in general includes physical contact between seedlings regardless of their pathological status (Plate 3.1I). But just before grafting, most of the infected leaves are removed so that only the four basal ones are left.

General nursery hygiene varied from one place to another. In some the grafting capes and other seedling debris are dispersed on walking paths between the seedlings (Plate 3.1 J&K) in other nurseries such materials are piled and buried or re-used leaving the nursery completely clean. In general non-hygienic nurseries had high levels of disease prevalence.

Fungicides applied for anthracnose management in each nursery are presented in Table 3.3. Four active molecules of different families are used at variable frequencies and concentrations depending on location throughout the country. Mancozeb is applied between 0.33 and 2.4 g/l, flint between 5 and 30 g/l, volcano between, 0.94 and 10 ml/l and finally bayfidan at 10ml/l. In most nurseries, fungicides are applied upon the appearance of the first symptoms. Only one case (Chiure) was recorded with continuous preventive applications of Volcano. It was observed however that application of fungicides is sometimes immediately followed by multi-pot irrigation.

Other potential facilitators of disease dispersion observed in the nurseries are pests. Leaf miners and Mosquito bugs were observed and their presence reported by all nursery personnel throughout the country. These pests are managed by application of insecticides (Cyhalotrin and cypermetrin) at variable doses and frequencies (Table 3.4). Nurseries are sprayed with insecticide almost every 11 days (Table 3.4). Nursery informants use mostly Karate (lambda cyhalotrin). Some call it lampo, others name it cialoti. The names in the table reflect actual pronunciation by the informants.

3.4.3. Spatial and temporal disease distribution in a nursery

3.4.3.1. Shading effect on spatial distribution

Cashew seedlings in Mozambique are grown under different types of shading material, arrangement or formats and density of coverage (Plate 3.2). In general we found no evident impact of these variations on disease incidence. However, at Gile, where the 50%

conventional polyethylene shading was combined with sprinkle aerial irrigation, high level of disease prevalence and mortality was observed. This was enhanced by the presence of infected cashew branches just above the shading net (Plate 3.2E).

3.4.3.2. Watering effect on spatial distribution

Watering method for most of the nurseries is either by watering-can (droplet type) or by polyethylene pipe (Plate 3.3 A&B). The pipe watering method can also be subdivided into droplet type and tape type (Plate 3.3 B&C) depending of the convenience for the operator. In general the shower type is multi-pot and rapid technique and therefore frequently adopted than the tape type which is a single-pot and slow. The real impact of each method on disease spatial spread could not assessed because the techniques were changed randomly. The frequency of irrigation varied depending on the rainfall frequency and intensity for each location. In the majority of nurseries however, the seedlings are irrigated twice a day (Fig.3.1). Again we observed no effect of the frequency variation on the prevalence of the disease.

3.4.3.3. Agro-ecological zones effect on spatial distribution

Out of all ten agro-ecological zones of Mozambique (Fig. 3.2), cashew was found to be cultivated in seven (R1, R2, R3, R5, R7, R8 and R9) of which R2, R3, R5, R7, R8 and R9 were covered by the present survey. Anthracnose incidence in nurseries across the surveyed zones is presented in Table 3.5. Agro-ecological zones R5 and R8 showed the highest incidence of the disease in the nurseries, 6.22 and 7.79% respectively (Table 3.5).

3.4.4. Temporal distribution

Cashew anthracnose incidence in nurseries over time (Fig.3.3) indicates that the disease is predominantly prevalent from January to April. Only in two provinces, Inhambane and Cabo Delgado the disease was reported to be prevalent between May and August. In Nampula, the disease prevalence was confined only between January and April.

Anthracnose prevalence over different agro-ecological zones indicates that regardless of the agro-ecological zone, the disease appears consistently between January and April (Fig.3.4).

In R2, the disease occurred throughout the year depending on the nursery. In R7 anthracnose incidence was observed between February and August while in R9 it occurred only over a short period between April and June (Fig.3.4).

3.5. General discussion

Disease surveys on cashew have been been adopted aiming at geographic mapping, assess the diversity of pathogens and more importantly to evaluate crop losses associated with a particular pathogen (Afouda *et al*., 2013). Previous survey on anthracnose prevalence was made on adult trees (Dhindsa and Monjana, 1984). The survey established the disease as widely distributed in Mozambique and therefore the need for chemical control was recommended. Our study is the first anthracnose survey conducted on cashew nurseries in Mozambique. It confirmed the wide distribution of disease throughout the country and provided evidence that Zambezia had the highest prevalence of the disease. Probably because the disease is known to be more severe under moderate humid and moderately warm conditions (Ohler, 1979; Ponte, 1984). Zambezia is the most humid province in the country (Faria and Da Mata, 1965; Anon, 1986). The peak of disease was reported to be from January to April. This period coincides with the most rainy and humid period in all parts of the country (Anon., 1986).

In agro-ecological zone R2, all the nurseries had anthracnose throughout the year. This zone characterized by rains with no strict season (Ritchie., 1996) in contrast to other regions like R8 in which the rain season ends in April. This suggests that additional measures for disease control may required in R2. Anthracnose yield reduction on cashew is estimated between 30 and 50% without chemical treatment (Ponte, 1884; Freire *et al*., 2002, Freire and Cardoso, 2003). But significant anthracnose damage occurs on cashew seedlings (Piteira, 1996). Our study, revealed up to 60% seedling mortality. There are no data available on extreme cases of disease incidence because control actions are usually taken immediately after the disease epidemics initiation.

The role of soil and seeds in disease transmission was not evident. However, in Brazil, under laboratory conditions, Lopez and Lucas (2002) noted that 33% of seedlings emerging from untreated cashew seeds had necrotic lesions on cotyledons, hypocotyls or epicotyl and associated these with a wide range of fungi that can saprophytically survive on seeds. Thus,

although the need for seed treatment would be better justified through a prior seed testing by a specialized laboratory, preventive measures are sometimes simply not taken due to economic or technical limitations.

Seedlings placed under or close to infected cashew canopies showed increased disease prevalence. Evidence of this was found (CHAPTER V) and thus this practice should be avoided. Incidence of anthracnose on rootstock seedlings was almost three times that on grafted seedlings. The microclimate in highly dense arrangement of rootstock plants plus synchronized production of young leaves may be more conducive for disease development compared to grafted seedlings with unsynchronized leaf production. In addition, removal of infected leaves, during the grafting process, reduces the probability of plant-to-plant transmission.

Findings from this survey may suggest that scions or rootstock do not necessarily transmit the *Colletotrichum* sp. to grafted seedlings. This because infected seedlings may have died long before sprouting and thus not assessed during the study. Subsequent infections on grafted seedlings may be coming from other aerial sources.

Increased rate of grafting success after treating the scions with a fungicide has been observed by our informants. It may be due to fungicide action against other microorganisms or endophytic pathogens e.g. *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. and *Phomopsis anacardii* Early & Punith. These pathogens affect the seedling during the healing of graft unions and they have been controlled by periodical disinfections of grafting knives and immersion of scions in a fungicide solution (Intini and Sijaona, 1983; Freire *et al*., 2002). The reported use of lemon juice, as disinfectant, instead of fungicides, requires further research.

Precautionary measures, management strategies and attitudes of the nursery workers regarding the disease were variable. Some nurseries were clean others were not, some did use pesticides others did not or they did it at concentrations below the recommended doses and at variable frequencies. Spraying potted soil with a fungicide not recommended for soil application, reveals lack of guidance and training. Therefore it would be important to produce a manual on cashew seedling pests and disease management and use it in a nationwide training program for all nurserymen.

References

Afouda, L.C.A., Zinsou, V., Balagoun, R.K., Onzo, A & Ahohuendo, B.C. 2013. Identification of cashew nut tree's (*Anacardium occidentale* L.) diseases in Benin. *Bulletin de la Recherche Agronomic du Benin (BRAB) 73:13-19*.

Anon. 1986. Atlas geografico. Volume 1. Segunda ediçao, revista e actualizada. Republica Popular de Moçambique. Ministerio da Educaçao. Maputo.

Anon. 1996. Zonas Agro-ecologicas e Sistemas de Producao. Programa de Investimento em Extensao Agraria. Ministerio da Agricultura e Pescas de Moçambique. Maputo.

Anon. 1998. Componente Producao, Plano Director do Caju. Instituto de Fomento do Caju (INCAJU). Maputo.

Anon. 2001. Mozambique Cashew Scenario. *Plant Horti. Tech. 2:12*.

Anon. 2005. Manual Pratico de Gestao do Viveiro de Producao de Mudas de Cajueiro. INCAJU, Delegacao Regional Norte. Sector Tecnico. Nampula.

Faria, J.M.R & Da Mata, L.A. 1965. Algumas notas sobre o clima de Moçambique. Seviço Meteorologico Nacional. Lourenço Marques.

Freire, F.C.O. & Cardoso, J.E. 2003. Doenças do cajueiro. Pages 192-225 *in*: Doenças de Fruteiras tropicais de interesse agroindustrial. Freire, F.C.O.; Cardoso, J.E. & Viana, F.M.P. (eds.). Embrapa Informação Tecnica. Brasilia.

Freire, F.C.O.; Cardoso, J.E.; dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21:489-494*

Holderness, M. 1996. Plant Pathology. International Course on the identification of fungi of Agricultural and Environmental significance. 11 August –20 September. IMI, CAB International. Egham.

Intini, M. 1987. Phytopathological Aspects of Cashew (*Anacardium occidentale* L.) in Tanzania. *International Journal of Tropical Plant Diseases 5:115-130.*

Intini, M. & Sijaona, M.E.R. 1983. Little Known Disease of Cashew (*Anacardium occidentale* L.) in Tanzania. *Revista di Agricoltura Subtropicale e Tropicale 77:421-429.*

Lopez, A.M.Q. & Lucas, J.A. 2002. Effects of plant defense activators on anthracnose disease of cashew. *European Journal of Plant Pathology 108:409-420*

Masawe, P.A.L. 2006. Tanzanian Cashew Cultivars. Selected Clones. Naliendele Agriculture Research Institute, Mtwara. 64 p.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do Cajueiro. Cultivar. Associação de Técnicos de Culturas Tropicais, Porto. 204 p.

Ohler, J. G. 1979. Cashew. Communication 71. Department of Agricultural Research. Koninklijk Instituut voor de Tropen. Amsterdam. 260 p.

Piteira, M.C.C. 1996. Pragas e doenças do Cajueiro. Setubal.

Piteira, M.C.C. & Rodrigues, Jr. C. J. 1999. *Colletotrichum gloeosporioides* Penz. of cashew (*Anacardium occidentale* L.). Morphocultural studies and pathogenicity tests using cross inoculations on other tropical fruits. Estação Agronómica Nacional, Oeiras (Portugal). Minutes of the 2nd Biennial Meeting of the Portuguese Phytopathology Society.

Ponte, J.J. 1984. Doenças do cajueiro no Nordeste Brasileiro. Documento 10. EMBRAPA-Departamento de Difusao de Tecnologia. Brasilia. 51 p.

Richie B., 1996. Practical techniques in plant pathology. A guide based on routine practice at plant pathology unit. International Course on the identification of fungi of Agricultural and Environmental significance. 11 August –20 September. IMI, CAB International. Egham.

Sundararaju, D. & Babu, PC.S. 1999. Recent research on management of neem mosquito bug on cashew. National Research Center for Cashew. Puthur (D.K.)-Karnataka.

Table 3.1: Percentage of anthracnose infected cashew seedlings per province Mozambique (survey conducted in 2007)

Province and SD	**Infection (%) and number of seedlings assessed**		**Total**
	Rootstock	*Grafted*	
Gaza	(2.21) 20240	(0.60) 43530	(2.81) 63770
SD	3.23	0.55	
Inhambane	(0.11) 16200	(1.13) 43500	(1.24) 59700
SD	0.59	1.47	
Zambezia	(24,67) 27850	(3.62) 30021	(28.29) 57871
SD	37.15	4.13	
Nampula	(0.51) 86750	(0.79) 226911	(1.30) 313661
SD	0.31	1.62	
Cabo delgado	(2.15) 24350	(0.64) 33327	(2,79) 57677
SD	3.75	0.74	
Total	(30.33) 175390	(7.10) 377289	(38.50) 552679
Mean (%)	6.22	1.57	
SD	22.31	2.58	

Out of ( ) = Total number of seedlings assessed; SD = Standard deviation.

Table 3.2: Anthracnose incidence means on cashew seedlings in different provinces, districts and locations of Mozambique (survey conducted in 2007)

Order No.	Province	Distrit	Location	Incidence on seedlings (%)		
				Rootstock	*Grafted*	*Mean*
1	Inhambane	Homoine	Inhamussua	1.03	0.19	0.30
2	Inhambane	Inharrime	Sede	1.22	*	1.22
3	Inhambane	Zavala	Maculuva	*	3.08	0.77
4	Inhambane	Vilanculos	Mapinhane	0.11	1.13	0.80
Mean				***0.11***	***1.13***	***0.8***
SD				***0.59***	***1.47***	***0.38***
5	Zambezia	Nicoadala	Sede	0.83	0.89	0.85
6	Zambezia	Mocuba	Sede	4.44	0.57	0.73
7	Zambezia	Namacurra	Sede	3.18	*	3.18
8	Zambezia	Mocuba	Sede	48.046	9.21	10.45
9	Zambezia	Maganja C	Sede	14.09	3.66	9.65
10	Zambezia	Pebane	Mulela	2.14	1.36	1.75
11	Zambezia	Gile	Sede	100	9.65	74.65
12	Zambezia	Ile	Mulevala	*	* *	
13	Zambezia	Namarroi	Sede	*	0	0.00
Mean				***24.68***	***3.62***	***12.66***
SD				***37.15***	***4.13***	***25.38***
14	Gaza	Xai-Xai	Chidsoquene	0.72	0.37	0.71
15	Gaza	Macia	Sede	0	0.01	0.01
16	Gaza	Majancaze	Chicleguene	*	1.47	1.47
17	Gaza	Chibuto	Maleisse	5.92	0.42	1.89
18	Gaza	Majancaze	Macuacua	*	0.72	0.03
Mean				***2.21***	***0.60***	***0.82***
SD				***3.23***	***0.55***	***0.84***
19	Maputo	Marracuene		*	* *	
20	Cabo Delgado	Chiure	Katapua	0.41	1.35	0.64
21	Cabo Delgado	Namuno		0	*	0.00
22	Cabo Delgado	Macomia	Napulupo	*	0	0.00
23	Cabo Delgado	Ancuabe	Sede	*	0	0.00
24	Cabo Delgado	Mueda	Sede	0.41	1.22	0.51
25	Cabo Delgado	Namuno	Naupe	7.77	*	7.77
Mean				***2.15***	***0.64***	***1.49***
SD				***3.75***	***0.74***	***3.09***
26	Nampula	Meconta	Nacavala	0.71	4.45	3.21
27	Nampula	Nampula	Rapale	*	0.3	0.30
28	Nampula	Mogincual	Liupo	0.2	0.01	0.01
29	Nampula	Angoche	Namitoria	*	0.01	0.01
30	Nampula	Mogovolas	Nametil	0.95	0.03	0.90
31	Nampula	Moma	Chalaua	0.31	0.33	0.33
32	Nampula	Meconta	Nacavala	0.398	0.43	0.42
Mean				***0.51***	***0.79***	***0.74***
SD				***0.31***	***1.62***	***1.13***
Overall Mean				6.22	1.57	4.42
Overall SD				22.31	2.58	12.68

* No plants available; SD=Standard deviation

Table 3.3: Fungicides applied on cashew seedlings in different locations of Mozambique (survey conducted in 2007)

No.	District	Fungicide	Active ingredient	Concentration	Interval (days)
1	Homoine	Mancozeb	Copper oxychloride	2 g/l	7
2	Inharrime	Mancozeb	Copper oxychloride	0.33 g/l	7
3	Vilanculos	Mancozeb	Copper oxychloride	2.4 g/l	21
4	Nicoadala	Flint	Trifloxystrobin	Dn	21
5	Mocuba	Anvil	Hexaconazole	Dn	Dn
6	Namacurra	Flint	Trifloxystrobin	Dn	Dn
7	Maganja da Costa	Flint	Trifloxystrobin	5 g/l	7
8	Xai-Xai	Flint	Trifloxystrobin	30 g/l	21
9	Macia	Flint	Trifloxystrobin	1 g/l	Dn
10	Manjacaze	Volcano	Hexaconazole	10 ml/l	Dn
11	Marracuene	Flint	Trifloxystrobin	7.5	15
12	Chiure	Volcano	Hexaconazole	0.94	7
13	Ancuabe	Volcano	Hexaconazole	Dn	Dn
14	Nampula	Bayfidan	Triadimenol	10 ml/l	30

Dn = The respondent does not know.

Table 3.4: Insecticides applied on cashew nursery seedlings in different districts and locations of Mozambique (survey conducted in 2007)

Order No.	District	Location	Insecticide		
1	Homoine	Inhamussua	Comercial name	Dosage	Interval (days)*
2	Inharrime	Sede			
3	Zavala	Maculuva	Cypermetrine	5ml/10l	7
4	Vilanculos	Mapinhane			*
5	Nicoadala	Sede			*
6	Mocuba	Sede			*
7	Namacurra	Sede			*
8	Mocuba	Sede			*
9	Maganja C	Sede			*
10	Pebane	Mulela	Cialoti	1.5ml/l	*
11	Gile	Sede	Karate	Dn	14
12	Ile	Mulevala	Lampo	120ml/12l	1
13	Namarroi	Sede			*
14	Xai-Xai	Chidsoquene			*
15	Macia	Sede			*
16	Majancaze	Chicleguene			*
17	Chibuto	Maleisse	Karate	50g/10l	14
18	Majancaze	Macuacua			*
19	Marracuene		Karate	75g/10l	15
20	Chiure	Katapua			*
21	Namuno		Karate	50ml/15l	7
22	Macomia	Napulupo	Karate	15ml/10l	*
23	Ancuabe	Sede			*
24	Mueda	Sede	Karate		*
25	Namuno	Naupe	Karate	5ml/5l	*
26	Meconta	Nacavala			*
27	Nampula	Rapale			*
28	Mogincual	Liupo	Karate	05g/l	14
29	Angoche	Namitoria	Karate	05ml/10l	21
30	Mogovolas	Nametil	Karate	05/10l	11
31	Moma	Chalaua	Karate	05g/1l	15
32	Meconta	Nacavala	insect killer	1ml/l	7
Mean					11.5
SD					5.52

Dn= The respondent does not know, SD= Standard deviation, * Data not available.

Table 3.5: Anthracnose incidence on cashew seedlings in different agro-ecological zones, districts and locations of Mozambique (survey conducted in 2007)

Agro-eco*	Order No.	Distrit	Location	Incidence on seedlings (%)		
				Rootstock	*Grafted*	*Overall*
R2	1	Homoine	Inhamussua	1.03	0.19	0.30
R2	2	Inharrime	Sede	1.22	*	1.22
R2	3	Zavala	Maculuva	*	3.08	0.77
R2	4	Vilanculos	Mapinhane	0.11	1.13	0.8
R2	5	Xai-Xai	Chidsoquene	0.72	0.37	0.71
R2	6	Macia	Sede	0	0.01	0.01
R2	7	Majancaze	Chicleguene	*	1.47	1.47
R2	8	Majancaze	Macuacua	*	0.72	0.03
R2	9	Marracuene		*	*	0
	Mean			**0.62**	**1.00**	**0.59**
	SD			*0.54*	*1.05*	*0.54*
R3	10	Chibuto	Maleisse	**5.92**	**0.42**	**1.89**
				n.a	n.a	n.a
R5	11	Mocuba	Sede	48.05	9.21	10.45
R5	12	Maganja C	Sede	14.09	3.66	9.65
R5	13	Pebane	Mulela	2.14	1.36	1.75
R5	22	Mocuba	Sede	4.44	0.57	0.73
	Mean			**17.18**	**3.70**	**5.64**
	SD			*23.82*	*4.04*	*5.11*
R7	15	Ile	Mulevala	*	*	*
R7	16	Namarroi	Sede	*	0	0
R7	17	Chiure	Katapua	0.41	1.35	0.64
R7	18	Namuno		0	*	0
R7	19	Namuno	Naupe	7.77	*	7.77
R7	20	Nampula	Rapale	*	0.3	0.3
	Mean			**2.73**	**0.55**	**1.74**
	SD			*4.37*	*0.71*	*3.38*
R8	21	Nicoadala	Sede	0.83	0.89	0.85
R8	23	Namacurra	Sede	3.18	*	3.18
R8	24	Gile	Sede	100	9.65	74.65
R8	25	Macomia	Napulupo	*	0	0.00
R8	26	Ancuabe	Sede	*	0	0.00
R8	27	Meconta	Nacavala	0.71	4.45	3.21
R8	28	Mogincual	Liupo	0.2	0.01	0.01
R8	29	Angoche	Namitoria	*	0.01	0.01
R8	30	Mogovolas	Nametil	0.95	0.03	0.90
R8	31	Moma	Chalaua	0.31	0.33	0.33
R8	32	Meconta	Nacavala	0.398	0.43	0.42
	Mean			**13.32**	**1.58**	**7.60**
	SD			*35.04*	*3.15*	*22.27*
R9	24	Mueda	Sede	**0.41**	**1.22**	**1.63**
				n.a	n.a	
	Mean			6.22	1.57	**7.79**
	SD			22.31	2.58	

R=Agro-ecological region, SD=Standard deviation, n.a. = Not applicable, * No data

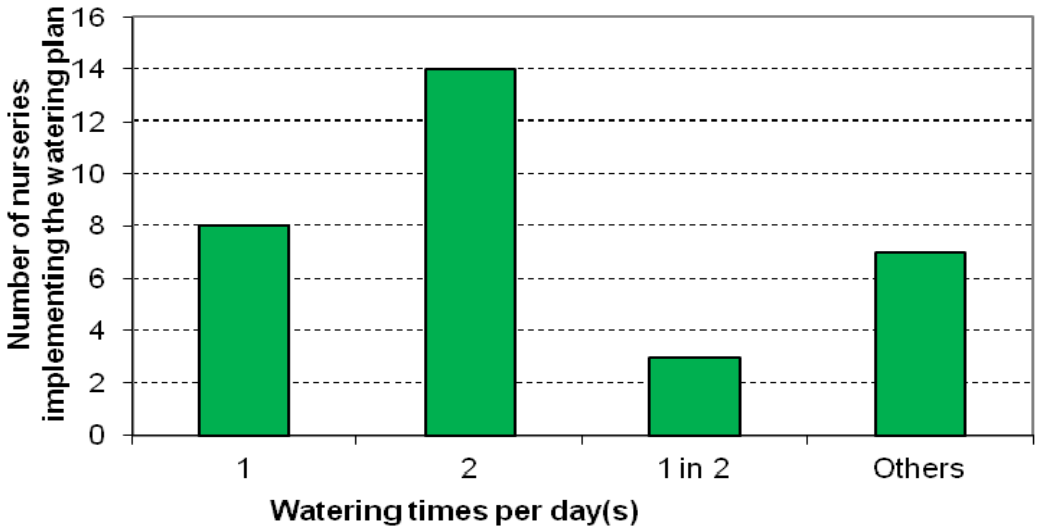


Figure 3.1: Watering frequency plan and number of executing nurseries in Mozambique (survey conducted in 2007).

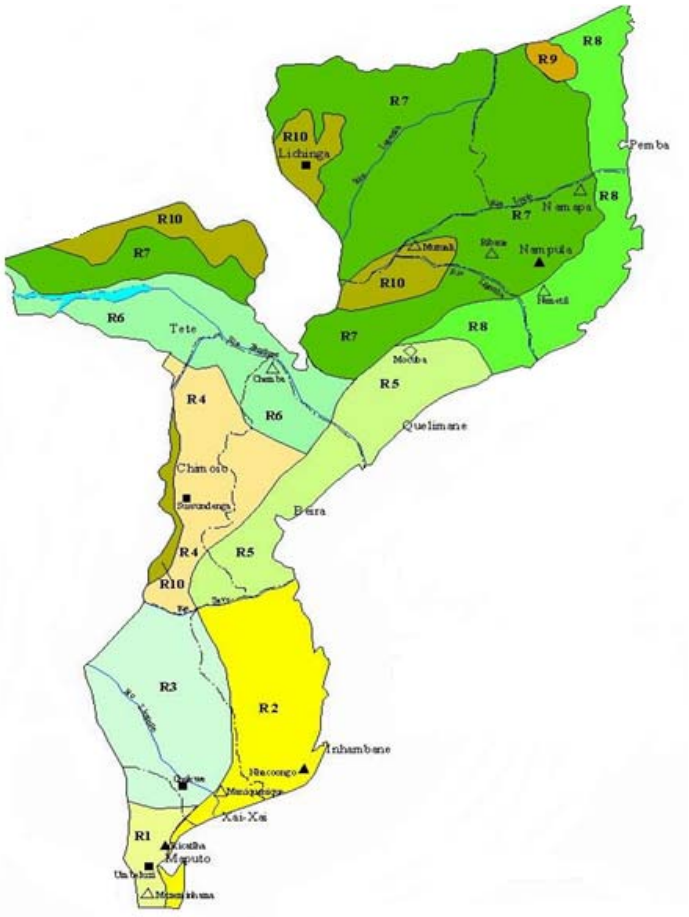


Figure 3.2: Map of agro-ecological zones (R1 to R10). Source: National Institute of Agriculture Research, 2009 (*personal communication*).

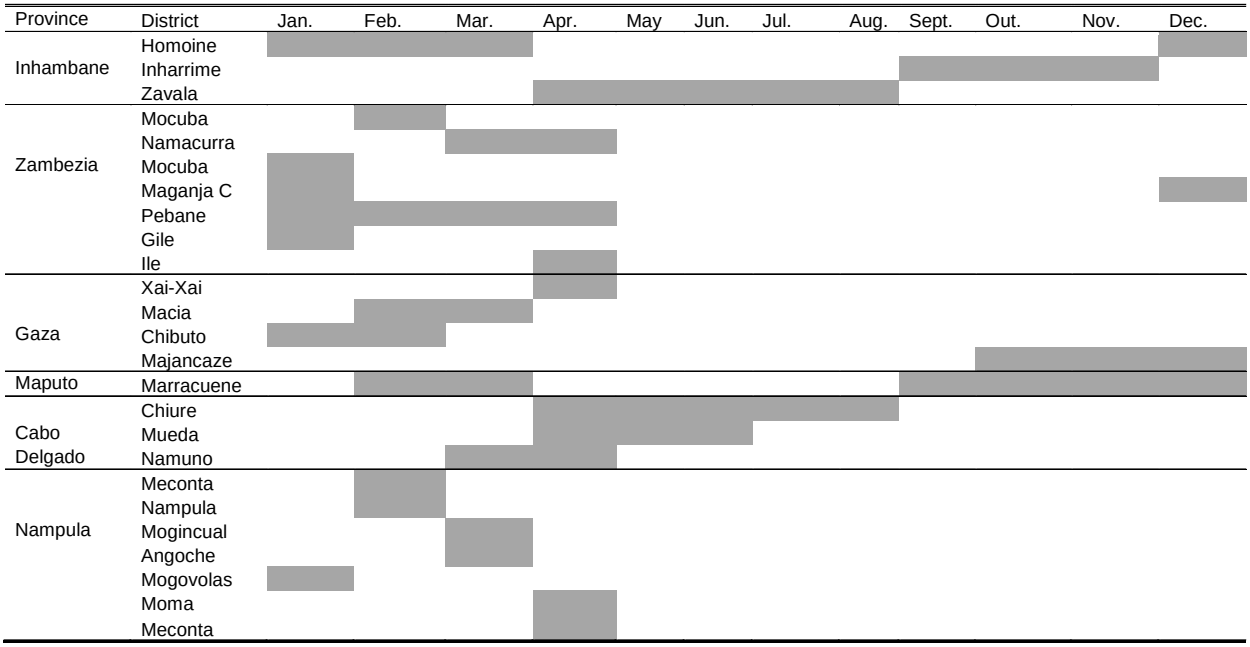


Figure 3.3: Anthracnose occurrence in cashew nurseries in different Provinces and Districts of Mozambique in 2007.

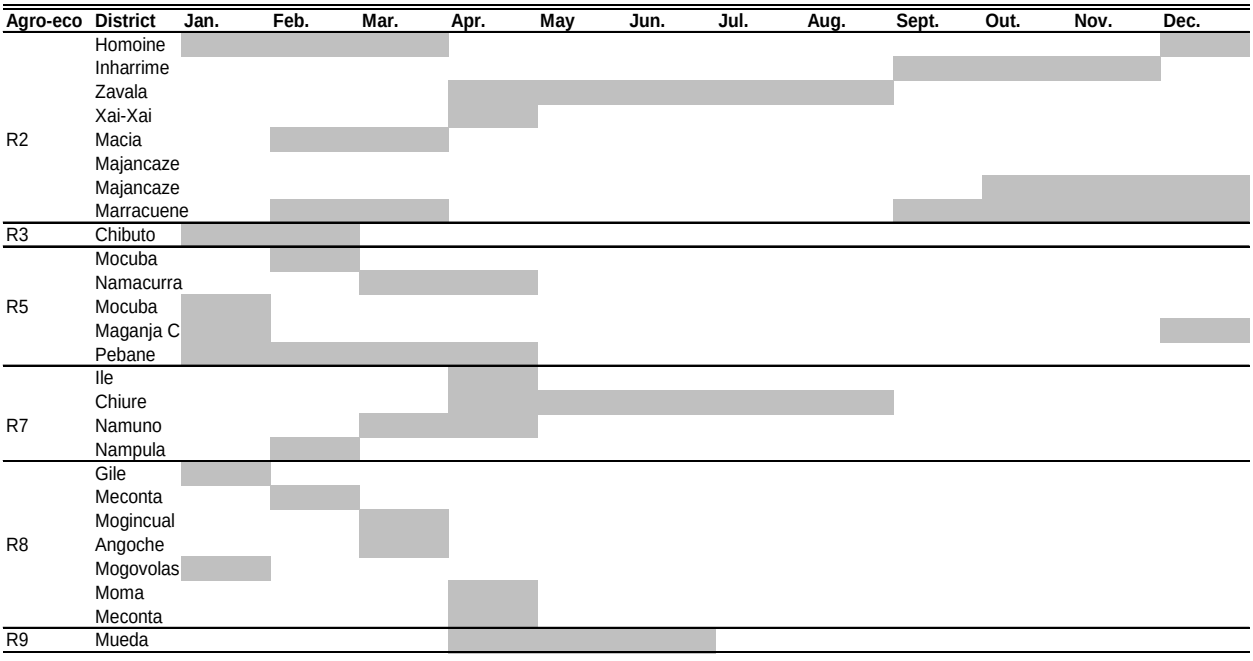


Figure 3.4: Anthracnose occurrence in cashew nurseries in different agro-ecological zones and districts of Mozambique in 2007; R = Agro-ecological region

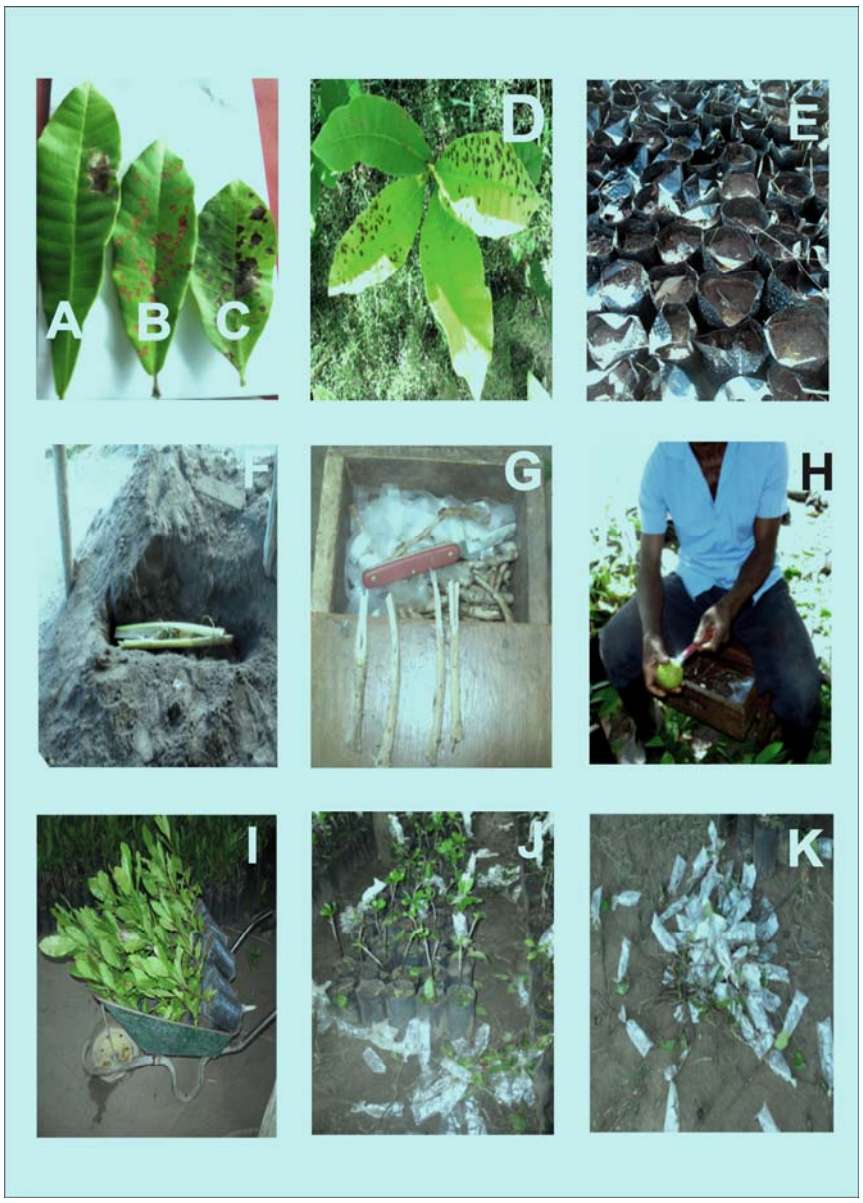


Plate 3.1: Cashew nursery pest and disease symptoms and common practices in Mozambique, 2007. Leaf miner damage (A), *Helopeltis* sp. damage (B), anthracnose necrosis (C), *Pestalotia* sp. leaf spots (D), fungicide sprayed pots (E), scions conservation (F), *Phomopsis* sp. infected scions (G), disinfection of grafting knife by lemon (H), seedling transportation (I), (J) grafting capes abandoned on ground together with health seedlings and (K); dead seedlings abandoned on passage.

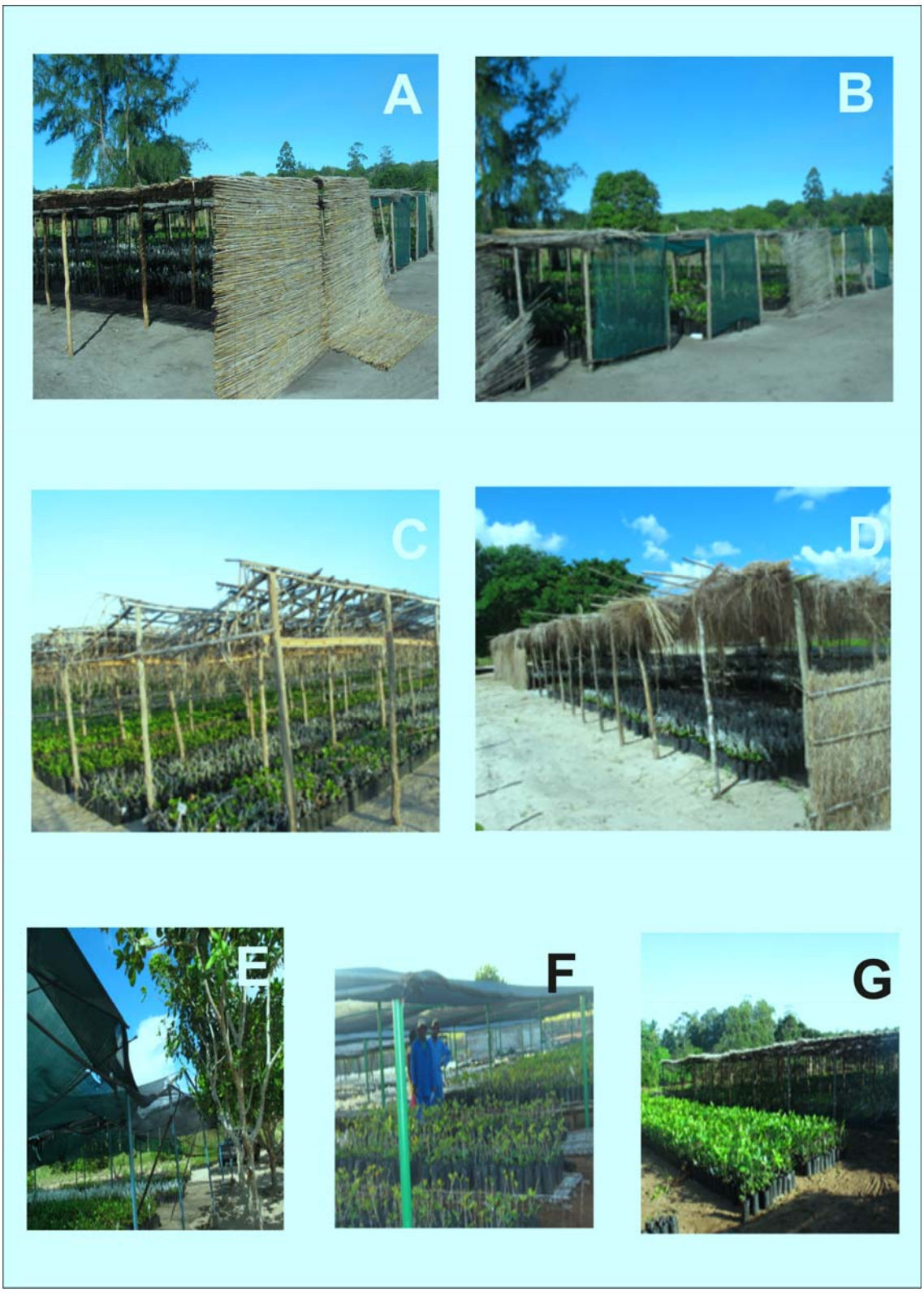


Plate 3.2: Variability of shading constructions in cashew nurseries: Mozambique, 2007. At Macuacua, Gaza Province (A), Chibuto, Gaza Province (B), Inharrime, Inhambane Province (C), Meconta, Nampula Province (D), Gilé, Zambezia province (E); Manjacaze, Gaza Province (F) and Chiure, Cabo Delegado Province (G).

Plate 3.3: Cashew seedlings watering techniques: Mozambique, 2007. Droplets released through a can (A) or by pressing with fingers at the end of a pipe (B), direct pot to pot release of water through a pipe tied to a stick (C) or not tied to a stick (D).

CHAPTER IV: LEAF AND FRUIT DISEASES OF CASHEW (*ANACARDIUM OCCIDENTALE* L.) IN MOZAMBIQUE AND THEIR PATHOGENS' SENSITIVITY TO FUNGICIDES

4.1. Abstract

Leaf and fruit samples were taken from the most important cashew growing provinces of Mozambique in a year period to determine the presence of pathogens. Symptoms associated with the pathogens in the field were described and pathogenicity tests conducted on detached cashew leaves and fruits. The main objective was to identify, among the commonly used fungicides, one with a large spectrum of action to control all the prevalent pathogens of cashew in Mozambique. Thus, isolates' sensitivity to different concentrations of the most commonly used fungicide on cashew was evaluated *in vitro*. Specific *in vitro* sensitivity to fungicide screening aiming at the causal agent of anthracnose was also undertaken. *Pestalotia* sp., *Colletotrichum* sp., *Phomopsis* sp. and *Fusarium* sp. were the most frequent isolates and they were all highly sensitive to hexaconazole which in turn revealed a better reduction of *Colletotrichum* sp. radial growth as compared to copper oxychloride and trifloxystrobin. The multiple pathogens inhibitor (hexaconazole) was recommended for field evaluation.

4.2. Introduction

A new leaf and nut blight (*Cryptosporiopsis* sp.) was described in Tanzania (Sijaona *et al*., 2005). But other leaf and nut diseases of cashew such as anthracnose, phomopsiose, pestalotiose, fusariose and cercosporiose are known from elsewhere (Freire and Cardoso, 1995; Freire *et al*., 2002; Intini and Sijaona, 1983). Similarities in disease symptoms make the diagnosis more complex for most of the extension officers. Despite that, simultaneous occurrence of pathogens with additive effect usually results in severe damage to the host tissue and complicates all management strategies. For instance, Ivey *et al*. (2004) reported on simultaneous occurrence of two species of *Colletotrichum* spp. from bell pepper while Whitelaw-Weckert *et al*. (2007) found three species on strawberries. To make the scenario

more complex, isolates from the same pathogen species may exhibit variable levels of aggressiveness towards the same host cultivar (Anon, 1999). Alternatively, each species may have different sensitivity to fungicides (Whitelaw-Weckert *et al*., 2007) as demonstrated in the case of almond *Colletotrichum gloeosporioides* Penz. and *Colletotrichum acutatum* J.H. Simmonds reaction to benomyl (Freeman *et al*., 2000). Therefore searching for suitable fungicides capable of targeting multiple pathogens at once is important for a cost effective disease control approach. In this context, the aims of this study were to (i) isolate and identify pathogenic fungi associated with cashew leaf and fruit spots, (ii) highlight their distinctiveness, (iii) conduct comparative pathogenicity tests and (iv) to compare sensitivity of the isolated pathogens against some commonly used fungicides in Mozambique.

4.3. Material and methods

4.3.1. Disease identification and prevalence

From each nursery, at least five symptomatic leaf samples were examined and collected into individual paper bags for isolation, within 48 hours, and identification of the pathogen associated with disease symptoms. More than 100 samples were collected from the Northern provinces of Zambezia (Pebane and Mocuba) and Nampula (Meconta) and Southern provinces of Gaza (Manjacaze), Inhambane (Inhamussua) and Maputo (Marracuene) in Mozambique. The present study was carried out in the phytopathology laboratory at Nampula Agricultural research Station, North East Zonal Centre of Mozambique Agricultural Research Institute (IIAM), from July 2005 to June, 2006.

The samples were surface sterilized and analyzed by cultivation on PDA and fungal isolation according to Anon. (1996) and Gonzalez and Sutton, (2004). Isolates of pathogens were recovered from leaf and nut spots by cutting a small section (5x5 mm) of the symptomatic area from the leading edge of young lesions, surface sterilize in 70% ethanol and culturing on potato dextrose agar medium (PDA, Biolab Diagnostics (Pty) Ltd, Wadeville, Gauteng, RSA), potato carrot agar (PCA) or sabouraud dextrose agar (SDA) Oxoid Ltd., Basingstoke, Hampshire, England) (Anon., 1996; Ivey *et al*., 2004). After 72 hours of incubation at laboratory temperatures 24+_1^{o}C, isolates were cultured by placing onto new plates a single plug cut from the fungal advancing edge of the infected section (Sanders and Korsten, 2003). Monoconidial cultures were produced for each isolate and maintained on SDA slants at 6 ^{o}C

and transferred every 30 days. Fungi isolated were identified to genus level based on the respective morphology of reproductive structures using dichotomic and illustrated keys per Anon[a] (1996).

Prevalence of the disease in nurseries was measured as percentage of seedlings with at least one leaf containing at least one typical necrotic spot. Grafted seedlings were evaluated separately from the rootstocks. Grafted but still uncovered or not sprouted seedlings were excluded. Mortality percentage was recorded.

4.3.2. Pathogenicity test

Isolates considered for pathogenicity test were those from the fungal genera known to cause diseases on cashew. These were identified based on their conidial morphology (Anon., 1996) observed under a binocular microscope (Axyoster Plus, Zeiss, Germany). Conidial suspension (ca 105 cfu/ml) was prepared from 10-15 days on SDA agar in Petri dishes. Actively growing mycelium was also used as small culture blocks. Immature detached cashew leaves, fruits (apples and nuts) were superficially disinfected in ethanol 70%, allowed to air dry and then inoculated with the conidial suspension or sterile distilled water (control), culture or just medium blocks (ca 2x2 cm). Both, pricking and smearing or just smearing techniques (Sijaona *et al*., 2005) were used for conidia inoculation. Culture blocks were placed on the host contacting the pathogens' mycelium to the disinfected surface. Inoculated detached organs were permanently incubated under high humidity conditions in tightly closed plastic boxes and incubated at 28 ^{o}C. Seedlings were inoculated by smearing technique and then covered with wetted plastic bags and maintained in the room for a day (Sijaona *et al*., 2005). The organs and seedlings were daily monitored for the occurrence of symptoms. Re-isolations were made to fulfill Koch's postulates (Anon., 1996).

4.3.3. Pathogen interactions on detached leaves

Pair-wise fungal combinations were made by mixing conidial suspensions immediately before inoculation or by placing two culture discs at the same inoculation site. Inoculated detached organs were incubated and monitored for symptom development and pathogen re-isolated as described before. .

4.3.4. *In vitro* fungicide screening

Autoclaved SDA was amended with suspensions of 0, 1, 1.5 and 2 times the recommended concentrations of the cashew fungicides commonly used in Mozambique: copper oxychloride, hexaconazole and trifloxystrobin. One ml of fungicide suspension was poured into the medium prior to its setting and gently mixed for uniformly. Control medium was not amended with fungicide. When the media had solidified, mycelial discs (4.0 mm diameter) from seven day old starter cultures were centrally plated onto three or four replicate Petri dishes (9 cm diameter) (Sanders *et al*., 2000; Vivekananthan *et al*., 2004). Fungicide amended and fungicide-free media were incubated under laboratory temperature (24+- 1^{o}C) (Karadimos *et al*., 2005) under daylight type fluorescent tubes (TL 40W/33RS) conditions (Sanders *et al*., 2000). The plates were arranged in randomized complete block design (Gomez and Gomez, 1984). Radial growth was measured daily using a ruler and data recording terminated only when fungal growth in at least one Petri dish had reached the edges. The experiment was repeated twice. Data were subjected to analysis of variance (ANOVA) and separation of growth means per concentration for each pathogen and fungicide by Tukey's test using the SAS GLM (SAS Institute, USA) statistical package.

4.4. Results

4.4.1. Symptom description and pathogens' isolated

All the interviewers were familiar with the symptoms presented in the questionnaire forms and could easily recognize them in both adult and young plants. However, sometimes similar symptoms caused by mosquito bug or necrotic lesions caused by *Pestalotia* sp., confused distinctive recognition of anthracnose. In close observation of these symptoms we noted that the pest-borne necrotic spots are reddish, translucent and concentrated along the main leaf vein (Plate 3.1A&B). Anthracnose necrotic spots occurred on inter-vein spaces, their margin is rather darkened than brown mainly towards the veins (Plate 3.1C). Spots caused by *Pestalotia* sp. were found in association with generalized seedling chlorosis and they are typically black in color (Plate 3.1D).

Isolations made at the laboratory revealed the presence of *Colletotrichum* sp. from most samples collected. From a single spot, two or more pathogens were sometimes isolated.

Colletotrichum sp. and *Pestalotia* sp. an association was commonly observed from leaf sample isolates.

The following symptoms were observed from field samples:

- On leaves collected from adult trees or seedlings, *Colletotrichum* sp. was isolated from the margins of necrotic, brown, large and wet areas. Sometimes numerous acervuli were observed on the abaxial part of the leaf laminae (Plate 4.1 A&D). It was also frequent to isolate *Colletotrichum* sp. and *Pestalotia* sp. from the same symptoms and occasionally *Colletotrichum* sp. in association with *Fusarium* sp.. Blackish depressed necrotic lesions were observed on apples and nuts (Plate 4.1C) or completely mummified greyish fruits were found in association with *Colletotrichum* sp. or *Colletotrichum* sp. and *Pestalotia* sp..

- *Pestalotia* sp. necrotic blackish spots were relatively small sometimes with halo and visible fungal powdery growth on the abaxial surface of mature leaves (Plate 4.1 J&K).

- *Phomopsis* sp. was isolated from blackened dying shoots. This regressive death of the tip of the branch sometimes affected also tender leaves or inflorescence (Plate 4.1N). *Phomopsis* sp. in association with *Fusarium* sp. was also found in these symptoms. No single case of sole Fusarium infection was observed from field samples.

- *Cryptosporiopsis* sp. infection was characteristically blackened or dark brown smooth nuts. No acervuli or dark margin, but sometimes, wet oily spot margins were commonly observed particularly under mist conditions of June and July (Plate 4.1 G&H).

4.4.3. Pathogenicity test and inoculation technique

All inoculation techniques reproduced disease symptoms on nuts, apples and both detached and seedling leaves (Plate 4.1.) Discs were very effective in producing symptoms on leaves within three days.

Pathogens' parallel inoculations, by pricking or smearing techniques, on immature cashew nuts and apples reproduced the following distinctive symptom:

- Depressed centre and raised darker margins on *Colletotrichum* sp. inoculated nut or apple samples (Plate 4.1 B, C&E). Smooth margins were on *Cryptosporiopsis* sp., *Fusarium* sp., *Pestalotia* sp. and *Phomopsis* sp. reproduced indistinct symptoms on young fruits of the test cultivar.

- Presence of white bright mycelium on inoculated leaves was always associated with *Pestalotia* sp. infection while a reddish brown color along the veins was consistently found in cases of *Fusarium* sp. infection. The presence of numerous fruiting bodies after 12 days was typical of *Phomopsis* sp. infection.

4.4.4. Pathogen interactions on host tissue

On young detached leaves, *Cryptosporiopsis* sp. was relatively fast in producing larger symptoms by the 3rd day, while the other pathogens produced smaller necrosis even by 5th day after inoculation. Mixing pathogens did not show any evidence of additive effect on symptoms development under the provided environment.

4.4.5. *In vitro* fungicide screening

4.4.5.1. On solid media

The mycelia of all tested pathogenic isolates was significantly sensitive to hexaconazole, regardless of the concentration used (Table 4.1). Mycelium radial growth of *Colletotrichum* sp. varied with type and concentration of fungicide used (Table 4.2). Significant differences were detected between mycelium growth on recommended concentrations and the un-amended controls for all fungicides except for copper oxychloride. On trifloxystrobin and copper oxychloride amended plates radial growth of the pathogen was not affected by increased concentration of the fungicide above the recommended dose (Table 4.2). On, triadimenol amended plates significant radial growth of the pathogen was recorded when the recommended concentration was doubled (Table 4.2). Both hexaconazole and triadimenol

fungicides at the tested concentrations did not inhibit radial growth of the *Colletotrichum* isolate on solid media (Table 4.2).

4.4.5.2. On liquid media

Conidia of all isolates immersed in any of the following fungicides, hexaconazole, triadimenol, trifloxystrobin and copper oxychloride did not produce colonies. Conidial germination and mycelium layer formation was observed only on fungicide un-amended controls. Similarly, mycelial discs placed in liquid fungicide amended medium, showed no growth. Mycelial growth was observed in un-amended controls (Plate 4.3).

4.5. General discussion

Distinctive anthracnose symptoms associated with *Colletotrichum* on cashew organs have been reported and illustrated by various authors (Ohler, 1979; Milheiro and Evaristo, 1994; Ferrão, 1995; Freire and Cardoso, 1995; Freire *et al*., 2002). A leaf and nut blight disease with similar symptoms particularly on leaves, was reported in Tanzania (Sijaona *et al*., 2005; Menge *et al*., 2013). Following our field observations of symptoms and confirmation by Koch's Postulates, as well as the isolation from similar symptoms of *Colletotrichum* by previous researchers in Mozambique (Carvalho and Mendes, 1958; Dhindsa and Monjana, 1984; Silinto, 2005), we therefore confirm the association of the observed symptoms of cashew anthracnose as caused by *Colletotrichum* sp.. Further support is provided by the absence of white spore masses which are found in nut blight as described by Sijaona *et al*. (2005).

In this study, *Colletotrichum* sp. and *Pestalotia* sp. were sometimes isolated from the same symptoms and occasionally *Colletotrichum* sp. and *Fusarium* sp. In Tanzania, *Pestalotia* attack on cashew was always observed in association with other pathogens and thus recommended for consideration in integrated control of cashew diseases (Intini & Sijaona, 1983). The occurrence of fungi complexes associated with cashew diseases has been reported also in Nigeria where *Lasiodiplodia*, *Pestalotia* and *Fusarium* species were isolated from the same symptom (Adeniyi *et al*., 2011). Similarly, on guava, *C. gloeosporioides* and *Pestalotia psidii* Pat. were found to be causing leaf spots (Williams and Bosland, 1988). On cranberry, *Colletotrichum acutatum* J.H. Simmonds and *Pestalotia vaccinii* (Shear) Guba are among the

most frequently recovered fungi from rotted fruits (Olatinwo *et al*., 2003). The occurrence of non-symptomatic infection of *Fusarium* may be associated with its endophytic or potential pathogen behavior as previously reported on *Copernicia prunifera* (Mill.) H.E. Moore (Freire & Bezerra, 2001). This is because when inoculated on detached leaves, under pathogenicity test conditions, the recovered *Fusarium* produced alone, typical symptoms of Fusariosis as described by Freire *et al*. (2002). The study also identified *Phomopsis* sp. in association with die-back of cashew shoots. The species could not be confirmed in this study. However, *Phomopsis anacardii* was described by Early and Punithalingam (1972) on cashew host. In neighboring Tanzania, the occurrence of cashew shoot die-back caused by *P. anacardii* has been reported (Intini & Sijaona, 1983).

All pathogens namely *Pestalotia* sp., *Phomopsis* sp., *Colletotrichum* sp. and *Fusarium* sp. were sensitive to hexaconazole at recommend dose. The sterol inhibitor fungicide restricted both conidial germination and mycelial growth. Hexaconazole has been applied against powdery mildew at intervals of 21 days, three to five times per cropping season (Uaciquete, 2004). Anthracnose however, under favorable conditions may require a higher frequency of applications, that is, at shorter intervals for effective chemical control (Prior *et al*., 1992). The present study highlighted the large spectrum effect of hexaconazole on cashew pathogens. This finding suggests that an increase in the frequency of hexaconazole application on cashew may result in multiple disease control.

When *Colletotrichum* sp. radial growth was evaluated *in vitro*, on three fungicides: Copper oxychloride, hexaconazole and trifloxystrobin, at different concentrations, only hexaconazole was effective in reducing mycelial growth at the recommended dose for powdery mildew control. There was an increase in mycelial growth when the dose of hexaconazole was tested at higher concentration. Formulated products contain adjuvants that can modify the activity of the fungicide or its properties and thus interfere with the activity of a fungicide (Banos *et al*., 2002; Karadimos *et al*., 2005).

Sensitivity of *Colletotrichum gloeosporioides* to copper oxychloride was practically not detected in the mycelial growth rate test method. The CV was very high, reflecting a low precision in measurements. The effect of adjuvants referred to before, combining with the presence of a blue color from copper ions and the irregularly fungal growth, i.e. uneven margins (Foster *et al*., 2003) made it difficult to precisely trace and measure the gray colored

mycelium. Copper based fungicides have been effectively used in field anthracnose management (Freire *et al*., 2002). Being a protectant fungicide its effect may be better expressed by lowering the proportion of germinating spores rather than suppression of post germination hyphal growth, the effect expected from curative fungicides (Xu and Butt, 1996).

The curative fungicide trifloxystrobin did not reduce the growth of *Colletotrichum* mycelium *in vitro*. In contrast, at double the concentration from that recommended for powdery mildew control, it stimulated very significantly the radial growth. The mode of action of strobilurin fungicides is by inhibiting mitochondrial respiration and thus confers them a large spectrum of activity (Sudisha *et al*., 2005). However, a relatively lower strobilurin sensitivity of fungal mycelium has been observed in other fungal species and this was associated with potential induction of alternative respiration mechanism (Karadimos *et al*., 2005).

The effectiveness of hexaconazole, demonstrated in this *in vitro* study, in addition to the already demonstrated efficacy against *Oidium anacardii* Noack the causal agent of powdery mildew (Smith *et al*., 1997) as well as relatively lower unit price, makes it potentially appropriate for cashew diseases management in Mozambique. Therefore field investigations were carried out with this fungicide (CHAPTER VI).

References

Anon. 1996. International Course on the Identification of fungi of agricultural and environmental significance. 11 August to 20 September, 1996. International Mycological Institute, Bekeham Lane, Egham, UK.

Anon. 1999. Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew (*Anacardium occidentale* L.). Summary reports of European Commission supported STD-3 projects (1992-1995), published by CTA. [online http://intership.cta.int/pubs/std/vol2/pdf/221/pdf] 111-116, March 25, 2009.

Adeniyi, D.O., Orisajo, S.B., Fademi, O.A., Adenuga, O.O., & Dongo, L.N. 2011. Physiological studies of fungi complexes associated with cashew diseases. *ARPN Journal of Agricultural and Biological Science 6:34-38*.

Banos, S.B., Necha, L.L.B., Luna, L.B. & Torres, K.B. 2002. Antifungal Activity of leaf and Stem extracts from various species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. *Revista Mexicana* de *Fitopatologia 20:8-12.*

Carvalho, T. & Mendes, O. 1958. Doenças de Plantas em Moçambique. Repartição de Sanidade Vegetal, Minerva Central, Lourenço Marques. 84 p

Dhindsa, P.P. & Monjana, A.M. 1984. Index of plant diseases and associated organisms of Mozambique. *Tropical Pest Management 30*:*407-429*.

Ferrão, J.E.M. 1995. O cajueiro (*Anacardium occidentale* L.). Instituto de Investigação Cientifica Tropical, Lisboa. 298 p.

Foster, H., Kanetis, L. & Adaskaveg, J.E. 2003. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus fungicide interactions. *Phytopathology 94*:*163-160*.

Freeman, S., Minz, D., Jurkevitch, E., Maymon, M. & Shabi, E. 2000. Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopathology 90:608-614*.

Freire, F.C.O. & Bezerra, J.L. 2001. Foliar endophytic fungi of Ceará State (Brazil): a preliminary study. *Summa Phytopathologica 27*:*304-308*.

Freire, F.C.O. & Cardoso, J.E. 1995. Doenças do cajueiro. Pages, 250-267 *in*: Cajucultura, modernas técnicas de produção. De Araujo, J.P.P. & da Silva, V.V. (eds). Embrapa-CNPAT, Fortaleza, Brasil.

Freire, F.C.O., Cardoso, J.E., dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21*: *489-494*.

Gomez, A & Gomez, R. 1984. Statistical procedures for agricultural research. 2nd Edition. John Wiley & Sons, London. 680 p.

Gonzaléz, E. & Sutton, T. B. 2004. Population diversity within isolates of *Colletotrichum* spp. Causing *Glomerella* Leaf Spot and Bitter Rot of Apples in Three Orchards in North Carolina. *Plant Disease 88: 1335-1340*.

Intini, M. & Sijaona, M.E.R. 1983. Little known diseases of cashew (*Anacardium occidentale* L.) in Tanzania. *Revista di Agricoltura Subtropicale* e *Tropicale 77*: *421-429*.

Ivey, L., Nava-Diaz, M.L. & Miller, S.A. 2004. Identification and management of *Colletotrichum acutatum* on immature bell peppers. *Plant Disease 88*: *1198-1204*.

Karadimos, D.A., Karaoglanidis, G.S. & Tzavella-Klonari, K. 2005. Biological activity and physical modes of action of Qo inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*. Crop *Protection 24*: *23-29*.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do Cajueiro. Cultivar. Associação de Técnicos de Culturas Tropicais, Porto. 204 p.

Olatinwo, R.O., Hanson, E.J. & Schilder, A.M.C. 2003. A first assessment of Cranberry fruit rot complex in Michigan. *Plant Disease 87*: *550-556*.

Prior, C., Elango, F. & Whitwell, A. 1992. Chemical control of *Colletotrichum* infection in mangoes. Pages 327-335 *in*: *Colletotrichum*: Biology, Pathology and Control. Bailey, J.A. & Jeger, M.J. (eds). British Society for Plant Pathology, Wallingford.

Sanders, G.M., Korsten, L. & Wehner, F.C. 2000. Survey of fungicide sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. *European Journal of Plant Pathology 106: 745-752*.

Sanders, G.M. & Korsten, L. 2003. Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Microbiological Research 158:143-150*.

Sijaona, M.E.R., Reeder, R.H. & Waller, J.M. 2005. Cashew leaf and nut blight-A new disease of cashew in Tanzania caused by *Cryptosporiopsis* spp. *Plant Pathology 55*:*576-576.*

Silinto, B.F. 2005. Influência de alguns factores ambientais no desenvolvimento da anthracnose nas folhas do cajueiro (*Anacardium occidentale* L.). Trabalho de Licenciatura. Faculdade de Ciências. Universidade Eduardo Mondlane, Maputo.

Smith, D.N., King, W.J., Topper, C.P., Mhando, H. & Cooper, J.F. 1997. Studies on spray deposition on cashew trees in Tanzania with reference to uses of fungicides to control *Oidium anacardii*. *Crop Protection* 16:*313*-322.

Sudisha, J., Amruthesh, K.N., Deepak, S.A., Shetty, N.P., Sarosh, B.R. & Shekar, S.H. 2005. Comparative efficacy of strobilurin fungicides against downy mildew disease of pearl millet. *Pesticide Biochemistry and Physiology 81: 188-197*.

Uaciquete, A. 2004. Epidemiology and Control of powdery mildew (*Oidium anacardii* Noack.) on cashew (*Anacardium occidentale* L.) in Mozambique. MSc dissertation in Plant Pathology. Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria.

Vivekananthan, R., Ravi, M., Saravanakumar, D., Kumar, N., Prakasam, V. & Samiyapan, R. 2004. Microbially induced defense related proteins against postharvest anthracnose infection in mango. *Crop Protection 23*:*1061-1067*.

Whitelaw-Weckert, M.A., Curtin, S.J., Huang, R., Steel., C.C., Blanchard, C.L. & Roffey, P.E. 2007. Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia. *Plant Pathology 56:448-463*.

Williams, P.H. & Bosland, P.W. 1988. Effect of foliar applications of fungicides on the phylloplane mycoflora and fungal pathogens of guava. *Journal of Phytopathology 123*: *52-62*.

Xu, X. & Butt, D.J. 1996. Tests of fungicides for post-germination activity against *Nectria galligena,* causal agent of canker and fruit rot of apple. *Crop Protection 15*: *513-519*.

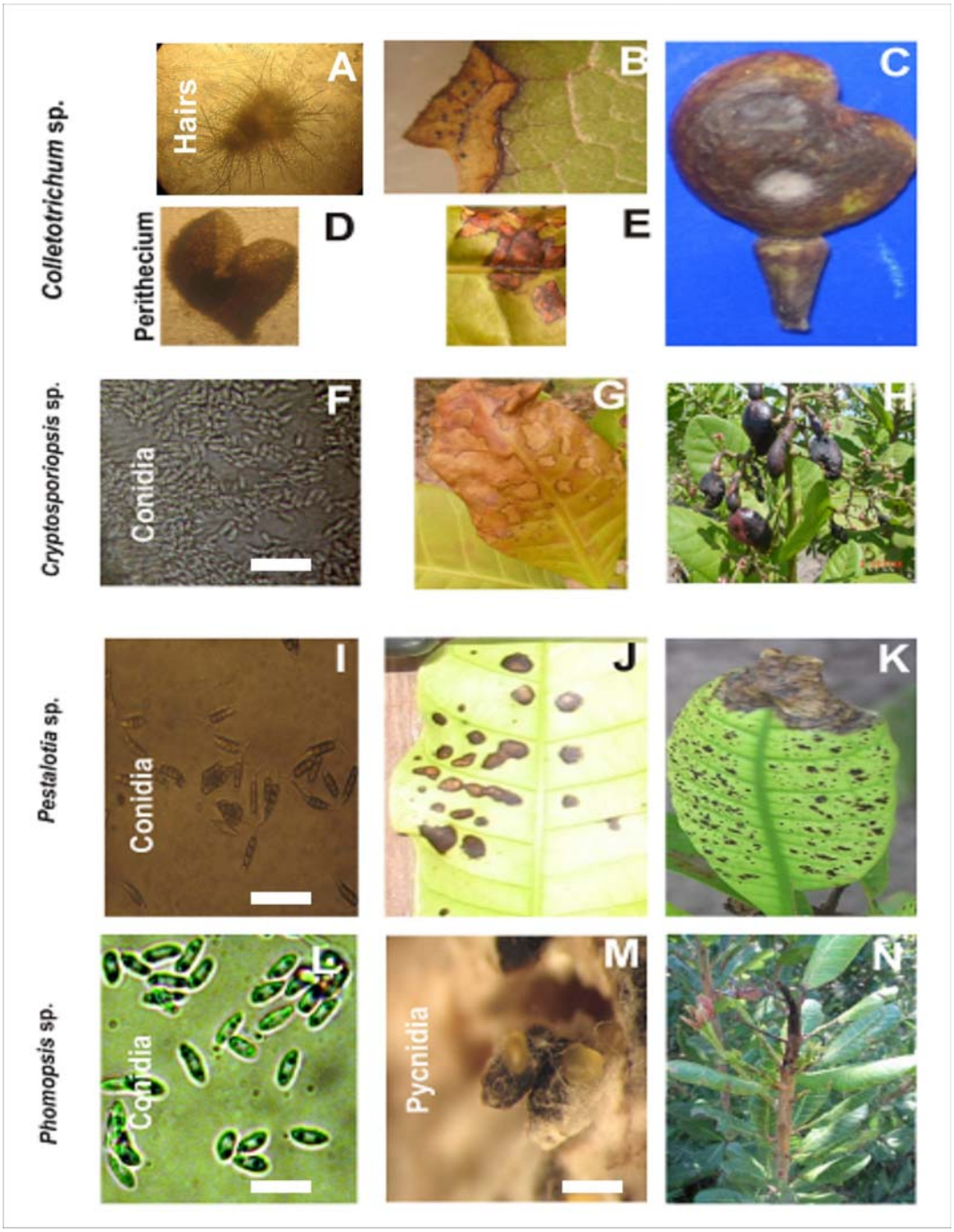


Plate 4.1: Cashew diseases: pathogen structures and distinctive symptoms on leaves, nuts and twigs. *Colletotrichum* sp.: A = Perithecium with setae (10 x magnified), B = necrosis on leaf with elevated margin, C= immature cashew nut with depressed spot full of mycelial growth, D = opened perithecium, E = multiple coalescent necrotic spots; Cryptosporiopsis sp.: F = Small hyaline conidia (400 x magnified), G = wet and blight necrotic spots on leaf laminae, H = nut blight and blackening symptoms; *Pestalotia* sp.: I = heteroconis conidia with conspicuous transversal septae (400x magnified), J = adaxial leaf spot and K= abaxial leaf spot, both on chlorotic leaves; *Phomopsis* sp.: L= binuclear greenish conidia (400 x magnified), M = elliptical pycnidia with oily exudate (5 x magnified) and N = Phomopsis dieback symptoms on a cashew twig. Horizontal bar = 5 µm.

Table 4.1: Mycelium radial growth means (mm) after 10 days of *Colletotrichum* sp., isolated from cashew leaves, on different concentrations of copper oxychloride, WP 85% triadimenol SC 5 % and trifloxystrobin WG 5%

Times the recommended Concentration	**Copper oxychloride** (4.25g/l)*	**Hexaconazole** (0.5g/l)*	**Trifloxystrobin** (1,5g/l)*
0.0	38.9 A	55.7 A	52.1 A
1.0	19.8 AB	38.1 B	45.0 B
1.5	3.3 B	37.6 B	44.0 B
2.0	3.6 B	28.8 C	41.5 B
SE	26.1	4.8	4.8
CV	132.1	8.75	9.50
Tukey's Prob.(α=0.01)	0.0008	<0.0001	0.0001

Means followed by the same letter vertically are not statistically different; * Recommended concentration of active ingredient. SE = Standard error ; CV = Coefficient of variance.

Table 4.2: Mycelium radial growth means (mm) after 10 days of *Colletotrichum* sp., isolated from cashew, on solid PDA plates amended with different concentrations of formulated copper oxychloride (WP 85%), Triadimenol (SC 5 %) and trifloxystrobin (WG 5%)

Times the recommended Concentration	**Copper oxychloride** (4.25g/l)*	**Triadimenol** (0.5g/l)*	**Trifloxystrobin** (1,5g/l)*
0.0	38.9 A	55.7 A	52.1 A
1.0	19.8 AB	38.1 B	45.0 B
1.5	3.3 B	37.6 B	44.0 B
2.0	3.6 B	28.8 C	41.5 B
SE	26.1	4.8	4.8
CV	132.1	8.75	9.50
Tukey's Prob.(α=0.01)	0.0008	<0.0001	0.0001

Means followed by the same letter vertically are not statistically different; * recommended concentration of active ingredient. SE = Standard error ; CV = Coefficient of variance.

CHAPTER V: EFFECT OF SEEDLING AGE AND DENSITY ON DEVELOPMENT OF CASHEW ANTHRACNOSE LEAF SYMPTOMS

5.1. Abstract

Cashew seedlings were grouped into three age categories and then placed under fully grown and infected cashew trees in a completely randomized design (CRD). Thus, seedlings were exposed to continuous natural inoculation by the anthracnose pathogen. Climatic parameters such as temperature, relative humidity and rainfall were retrieved from a local meteorological station. Daily evaluation of disease development was made from 24 hours (zero rating) onwards. Climatic conditions were favorable for disease development. On the youngest seedlings, symptom development was delayed one day compared to that on the older ones. After the appearance of symptoms, for four consecutive days, the level of leaf disease incidence and severity was consistently lower on the youngest seedlings. Therefore the application of fungicides on seedlings is recommended only after four days of exposure to inoculum and for seedlings with six or more leaves. In another trial, naturally inoculated seedlings were placed in a CRD experiment with five treatments: 0, 15, 30, 45 and 60 cm spacing between pots to provide different canopy densities. The proportion of leaves revealing anthracnose symptoms (% incidence) and respective severity (0-6 scale) were assessed every 48 hours. The frequency of wet leaves at specified times were recorded. Leaf wetness was highly reduced by increasing seedling spacing by at least 15 cm. It was recommended that prophylactic application of fungicides on cashew seedlings should commence only from the phenological stage of six leaves and as the seedlings grow, spacing must be widened to minimize both plant-to-plant inoculation and locally conducive micro-climatic conditions for infection. This approach can reduce the cost and risk of transplanting infected cashew seedlings. The natural inoculation method adopted in this experiment proved suitable for use in fungicide/cultivar screening programs.

5.2. Introduction

Anthracnose (*Colletotrichum gloeosporioides* Penz.) is the most common disease of cashew seedlings (Junior and Chaves, 2002). At least ten hours of air saturation (Freire *et al*., 2002) or four hours of wetness (Ohler, 1979) are required for *Colletotrichum* infection to take place. The water is important in dilution of gelatinous self-inhibitory conidial substances (Freire and Cardoso, 2002) and relative humidity is the most important factor during the infection process (Ohler, 1979, Dodd *et al*., 1991; Freire and Cardoso, 2002).

Anthracnose symptoms are observed mainly on aerial parts of the plant (Ohler, 1979) and more frequently on the leaves (Freire and Cardoso, 2003). Lesions are initially water-soaked and later become orange brown to light reddish with age and sporulation of the fungus (Freire *et al*., 2002). In cases of a severe attack defoliation may occur (Ponte, 1984) or even seedling mortality (Milheiro and Evaristo, 1994; Ferrão, 1995; Freire and Cardoso, 1995).

To avoid losses in the nurseries, the disease is mainly controlled by prophylactic use of fungicides (Freire *et al*., 2002). But, because there has been a build-up of resistance by the pathogen (Korsten *et al*., 1995; Dik *et al*., 1998) and limited availability of new fungicides (De Jager, 1999). The search for alternative non-chemical methods has been required (Korsten *et al*., 1995; Dik *et al*., 1998; De Jager, 1999). Thus, induced resistance has been demonstrated on cashew (Lopez and Lucas, 2002) but its wide use is limited by the narrow safety margin between effective and phytotoxic rates (Kessman *et al*., 1994).

It is well known however, that appropriate selection of plantlet or organ age or position enhances accuracy in screening programs: Contrasts in sensitivity to a pathogen, may be erroneously attributed to differences between genotypes or treatments if arbitrary selection is made (Visker *et al*., 2003). This is because plants resist pathogen infection through age related structural and chemical mechanisms. Natural defense mechanisms are poorly developed in immature tissues. These must either be preformed (cuticle and cell wall) or induced upon infection (reactive oxygen species, cell wall strengthening, phytoalexin biosynthesis and accumulation of pathogenesis–related defense proteins respectively (Rivera *et al*., 2002; Romero *et al*; 2008). In this study we aimed at reducing seedling cashew anthracnose (*C. gloeosporioides*) incidence and severity through seedling age and canopy density manipulation

5.3. Material and methods

Two trials were undertaken at Nassuruma National Cashew Research Center in the North East Province of Nampula, Mozambique, between April (week 14) and June (week 25), 2006. Daily climatic data collected from the nearest (one kilometer) Nassuruma Station included rainfall, temperature and relative humidity. These were later processed in excel spreadsheet to retrieve weekly means that were plotted graphically.

5.3.1. Effect of seedling phenological age on anthracnose disease development

Seeds were collected from a highly disease susceptible clone 11.9PA and potted into 15x28 cm plastic bags. Seedlings were commercially grown in covered greenhouse. To assess the effect of seedling phenological age, anthracnose asymptomatic seedlings were visually categorized into three groups, corresponding to different numbers of leaves. Twenty seedlings of each phenological age category were placed underneath severely infected tree canopies to induce natural inoculation. The seedlings were placed in five replicates of four plants per age category and positioned the same way in four locations north, south, east and west under each tree canopy (Plate 5.1). Seedlings were irrigated by pouring water directly, roughly half a liter, into each bag twice per day. The experiment was designed as a completely randomized design (CRD) (Petersen, 1994). The proportion of leaves revealing anthracnose symptoms (% incidence) and respective severity were assessed using an illustrated 0-6 leaf scale (Appendix 5) every 48 hours until most of the leaves (95%) in one of the treatments had developed visible disease symptoms.

5.3.2. Effect of seedling spacing on anthracnose disease development

To evaluate the effect of seedling density, 120 vegetatively uniform seedlings (clone 11.9PA), grown as described before, at the five leaf phenological stage were selected. The seedlings were later placed under the canopy of an anthracnose infected adult cashew tree (clone 11.9PA) for three days to naturally stimulate inoculation. After this period the seedlings were taken out from underneath the canopy and arranged experimentally in a four replicate CRD (Petersen, 1994). Five spacings were considered treatments: 0, 15, 30, 45 and 60 cm between

pots. Each treatment consisted of twelve seedlings positioned in a square, so that four of them were centered and surrounded by the others.

As previously described, total number of leaves, proportion of symptomatic leaves (incidence) and respective severity were assessed every 48 hours only on the four centrally located seedlings. In addition, leaf wetness on leaf laminae was visually evaluated hourly from five to nine a.m. every day. A discrete yes or no scoring system was adopted where "yes" meant that the leaves were wet and "no" meant that the leaves were dry.

Through the observation period, the last scoring hour was considered to be the one in which all leaves were considered dry. Therefore for comparative analysis only data from the previous hour were used. Actual treatment differences were measured by comparing the frequency of yes responses from the rating form. Thus, treatments whose seedlings remained wet, exhibited a prolonged wetness period for an additional one hour compared to the others. Frequency averages per treatment were computed and graphically plotted in Microsoft Excel. The higher the frequency of prolonged wetness the higher the cumulative period in which seedlings at certain spacing remain wet.

For the seedling age trial, incidence and severity data were statistically analyzed using GenStat (2003) for windows program. One-way analysis of variance (ANOVA) was used to test for differences between the three (young, intermediate and old) seedling ages. Young seedlings were defined as those with six or less leaves, intermediate seedlings with 6 to 14 leaves and the old ones with 15 or more leaves. The data was acceptably normal with heterogeneous treatment variances, thus age means were separated using Fishers protected t-test least significant difference (LSD) at the 1% level of significance (Snedecor and Cochran, 1980).

5.4. Results

5.4.1. Disease favorable climatic conditions

During the experimental period the climatic parameters were as described below:

Temperatures varied from a weekly mean minimum of 15°C to a weekly mean maximum of 31°C. Mean temperature ranged between 22 and 26°C (Fig.5.1). Air relative humidity weekly means were 80 to 90% at night, 60 to 80% in the mornings and 45 to 72% in the afternoons (Fig.5.1). There were showers most weeks except for weeks 19, 20, 23, 24 and 25 (Fig.5.1). In summary, the prevailing climatic conditions were not restrictive for disease symptoms to develop.

5.4.2. Effect of seedling leaf age

The data indicates that at the beginning of the trial, the age difference was clearly marked by highly significant difference on the number of leaves (Table 5.1).

All seedlings were disease free for one day after they had been placed under the infected tree. The first appearance of anthracnose disease symptoms (incidence) was observed on intermediate and old seedlings two days later (Table 5.1). By the third day, the disease was also recorded on the youngest seedlings representing a one-day delay in relation to other phenological stages.

When the youngest seedlings acquired one additional leaf, during the period from the second to the third day (Table 5.1), highly significant differences on anthracnose incidence as well as severity were registered (Table 5.1). These differences were not significant from the 4th day onwards. However, incidence was significantly lower on the youngest seedlings as per the last scoring day data (Table 5.1).

The mean number of leaves on the youngest seedlings increased two units (five in day 1 to seven in day 10). However these remained always significantly less than the number of leaves on the intermediate and old seedlings which did not increase almost throughout the whole experimental period (Table 5.1).

Anthracnose incidence under the described conditions, increased from zero to a maximum mean of 75-95% while the severity reached a maximum mean of 1.2 and 1.3 (less than 1% leaf area necrotic) depending on the age of the seedlings (Table 5.1).

5.4.3. Effect of seedling spacing

Observations on leaf wetness were made over twenty days of which thirteen had mist and the frequency of days with prevailing leaf wetness per spacing is presented in Fig. 5.2.

Prolonged wetness was observed with highest frequency on seedlings whose pots were directly next to each other (0 cm between seedlings). By moving the seedlings just 15 cm away from one another, the frequency of prolonged wetness was reduced almost three times and so on at 30, 45 and 60 cm (Fig.5.2). It can therefore be assumed that seedlings planted closely will remain wetter for longer periods of time.

5.5. Discussion

Differences of seedling age resulted in differential sensitivity to anthracnose disease. Thus, pathogen incubation period was 48 hours on "intermediate" and "old" seedlings. Symptom development has been observed within 48 hours under laboratory conditions (Silinto, 2005). In addition, *Colletotrichum* species are known to form appressoria within 6 to 12 hours after conidia germination (Cardoso and Freire, 2002). Although the present study supports the findings by previous researchers, (Cardoso and Freire, 2002; Silinto, 2005) surprisingly, on "younger" seedlings (less than six leaves), symptoms were delayed by 24 hours as compared to those on seedlings with more than six leaves. Similarly, delayed expression of symptoms has been observed on black walnut (*Juglans nigra* L.) seedling anthracnose pathosystem. The apparent (transitory) resistance of immature leaves was primarily due to leaf expansion during the period between inoculation and appearance of symptoms associated with high trichome density that impeded the laying of conidia on the surface. The duration of latency, expresses resistance or varietal tolerance on coffee and *Himeleia vastatrix* Berk. And Br. disease complex (Eskes and Toma-Braghini, 2006). In the cashew anthracnose pathosystem, the significance of latency on host tolerance requires further investigations.

Climatic conditions during the experimental period were appropriate for disease development (Fig.5.1). Warm and humid conditions, characterized by air saturation during the night and mornings were observed. These results are in conformity with the climate of Nampula area as characterized by Faria and Da Mata, (1965) for the study period. The period coincides with the reproductive cashew flush (Milheiro and Evaristo, 1994; Appendix 2). At the nursery, many seedlings awaited for the rain season before transplanting (November to March). In this period if properly watered, seedlings grow vegetatively. Combined, favorable environmental conditions and suitable host phenological phase, made it favorable for the disease to develop into epidemics. Previous other authors (Ohler, 1979; Freire and Cardoso, 2002) have reported on the suitability of similar conditions for increased anthracnose incidence and severity on cashew.

In this trial, a high level of anthracnose incidence was found on leaves (95%) contrasting with a low severity rating of less than 1%. This opposes the finding from adult cashew trees where anthracnose increases rapidly in both number (incidence) and size (severity) of lesions whenever climatic conditions and host phenology are favorable (Cardoso *et al*., 1999).

Initial four leaves were almost permanently dull and developed relatively small lesions upon *Colletotrichum* infection when compared to better developed leaves. Cashew leaf development takes 21 days to reach maturity or full green color (Ohler, 1979) but the initial four take less. In addition, Sijaona *et al*., (2001) noted that during seedling leaf development a dull and a shiny area can be distinguished. Shiny area was associated with gradual cuticle formation and thus found to be more susceptible to powdery mildew disease. Furthermore, we observed that the first four leaves are perpendicularly inserted to the stem allowing relatively lower periods of wetness or surrounding air saturation due to fast evaporation. Contrarily, the subsequent leaves were oblique to the stem and therefore exhibited propensity towards harvesting and retaining more wetness and therefore induce fungal spores to germinate.

Seedlings with more than six leaves developed numerous anthracnose lesions faster because the more the seedlings grew the more susceptible tissue was produced. Similar observations were made on adult trees, where anthracnose epidemics were found to reach peaks in conjunction with high levels of leaf flush (Cardoso *et al*., 1999).

We found from this experiment that high density of seedlings supported prolonged leaf wetness. Observations on adult trees revealed that such conditions are highly conducive to infection by anthracnose (Ohler, 1979; Ponte, 1984; Freire and Cardoso, 1995) and pruning or providing a wide spacing between plants at planting is recommended (Ikisan, 2000).

In conclusion, the study demonstrates that the disease intensity was reduced through seedling manipulation and thus minimizes the use of fungicides. High seedling density, resulting from both lower spacing and high leaf number, increase prolonged cumulative wetness and subsequent plant propensity to infection by anthracnose pathogen. It is therefore recommended that under circumstances of prevalent anthracnose, seedling spacing must be adjusted as they grow to minimize both plant-to-plant inoculation and favorable infection micro-climate. Since seedlings with six or fewer leaves showed lower incidence and severity over four days of exposure to the pathogen, it can also be recommended that preventive applications of fungicides on cashew nurseries should be initiated only from the emergence of leaf number seven. A natural seedling inoculation method was used for the first time on cashew-anthracnose pathosystem and proved to be effective. If combined with high density placement of seedlings, it can be used in fast screening programs for fungicides or cultivars.

References

Cardoso, J.E., Cavalcanti, J.J.V., Cavalcante, M. De J.B, Aragão, M. Do L. & Felipe, E.M. 1999. Genetic Resistance of dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mould and angular leaf spot. *Crop Protection 18: 23-27.*

De Jager, E.S. 1999. Microbial ecology of the mango flower, fruit and leaf surfaces. MSc. (Agric) thesis. University of Pretoria, Pretoria.

Dik, J.; Verhaar, M.A. & Belanger, R.R. 1998. Comparison of three biological control agents against cucumber powdery mildew (Sphaerotheca fuliginia) in semi-commercial scale glasshouse. *European Journal of Plant Pathology 104: 413-423*.

Dodd, J.C., Estrada, A., Matcham, J., Jeffries, P. & Jeger, M.J. 1991. The effect of climatic factors on Colletotrichum gloeosporioides, causal agent of mango anthracnose in the Philippines. *Plant Pathology 40:1-8*.

Eskes, A.B. & Toma-Braghini, M. 2006. The effect of leaf age on incomplete resistance of coffee to *Hemileia vastatrix*. Netherlands Journal of Plant Pathology. CAB abstract.

Faria, J.M. da R. & Da Mata, L.A. 1965. Algumas notas sobre o Clima de Moçambique. *Serviço Meteorologico de Moçambique 22:1-8.*

Ferrão, J.E.M. 1995. O cajueiro (*Anacardium occidentale* L.) Ministerio do planeamento e da Administração do territorio. Secretaria de Estado da Ciencia e Tecnologia. Instituto de Investigação Cientifica tropical. Lisboa. 298 p.

Freire, F.C.O. & Cardoso, J.E. 2003. Doenças do cajueiro. Pages 192-225 *in*: Doenças de Fruteiras tropicais de interesse agroindustrial. Freire, F.C.O.; Cardoso, J.E. & Viana, F.M.P. (eds.). Embrapa Informação Tecnica. Brasilia.

Freire, F.C.O., Cardoso, J.E., dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21:489-494*

Freire, F.C.O. & Cardoso, J.E. 1995. Doenças do cajueiro. Pages 249-267 *in*: Cajucultura: Modernas Tecnicas de Producao. De Araujo, J.J.P. & da Silva, V.V.(eds.). EMBRAPA-CNPAT. Fortaleza.

Ikisan, 2000. Cashew. Crop Information. http://www.Ikisan.com. January 27, 2008.

Junior, A.T.C. & Chaves, J.C.M. 2002. Produção de mudas de cajueiro. Pages 55-73 *in*: Caju produção. Aspectos tecnicos. Barros, L. De M. (ed.) EMBRAPA. Mapa. Brasilia.

Kessmann, H., Staub, T., Hofmann, C., Maetze, T., Herzog, J., Ward, E., Uknes, S. & Ryals, J. 1994. Induction of systemic acquired resistance by chemicals. *Annual Review of Phytopathology 32:439-459*.

Korsten, L., De Jager, E.S., De Villiers, E.E., Kotzé, J.M. & Wehner, F.C. 1995. Evaluation of epiphytes isolated from avocado leaf and fruit surfaces for biocontrol of avocado postharvest diseases. *Plant Disease 79: 1149-1156*.

Lopez, A.M.Q. & Lucas, J.A. 2002. Effects of plant defense activators on anthracnose disease of cashew. *European Journal of Plant Pathology 108: 409-420*.

Menge, D., Makobe, M.N., Shomari, S. & Tiedemann, V. 2013. Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. Causing leaf and nut blight (*Anacardium occidentale* L.). *International Journal of Advanced Research 1:26-38*.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do cajueiro. Cultivar. Associação de Tecnicos de Culturas Tropicais, Porto. 204 p.

Ohler, J. G. 1979. Cashew. Communication 71. Department of Agricultural Research. Koninklijk Instituut voor de Tropen. Amsterdam. 260 p.

Payne, R.W. 2003. GenStat® for Windows® (7th Edition)-Introduction. VSN International. 56 p.

Petersen, R. G. 1994. Agricultural field Experiments design and analysis. Maccel Dekker, Inc. New York. 409 p.

Ponte, J.J. 1984. Doencas do cajueiro no Nordeste Brasileiro. Documento 10. EMBRAPA-Departamento de Difusao de Tecnologia. Brasilia. 56 p.

Rivera, M.E.; Codina, J.C.; Olea, F.; De Vicente, A. & Perez-Garcia, A. 2002. Differential expression of *ß-1,3*-glucanase in susceptible and resistant melon cultivars in response to infection by *Sphaerotheca fusca*. *Physiology and Molecular Plant Pathology 61:257-265.*

Romero, D.; Rivera, M.E., Cazorla, F.M.; Codina, J.C., Fernandez-Ortuno, D., Torés, J.A., Peréz-Garcia, A. & De Vicente, A. 2008. Comparative histochemical analyses of oxidative burst and cell wall re-enforcement in compatible and incompatible melon-powdery mildew (*Podosphaera fusca*) interactions. *Journal of Plant Physiology 165:1895-1905.*

Sijaona, M.E.R., Clewer, A., Maddison, A. & Mansfield, J.W. 2001. Comparative of powdery mildew development on leaves, seedlings and flower panicles of different genotypes of cashew. *Plant Pathology 50:234-243*.

Snededcor, G.W & Cochran, W.G. 1980. Statistical methods (7th Edition). Ames: Iowa State University Press. 807 p.

Visker, M.H.P.W., Keizer, L.C.P., Budding, D.J., Van Loon, L.C., Colon, L.T. & Struik, P.C. 2003. Leaf position prevails over plant age and leaf age reflecting resistance to late blight in potato. *Phytopathology 93:666-674*.

Plate 5.1: Layout of the trial on age related natural infection of cashew seedlings by *Colletotrichum gloeosporioides*, at Nassuruma, Mozambique in 2006.

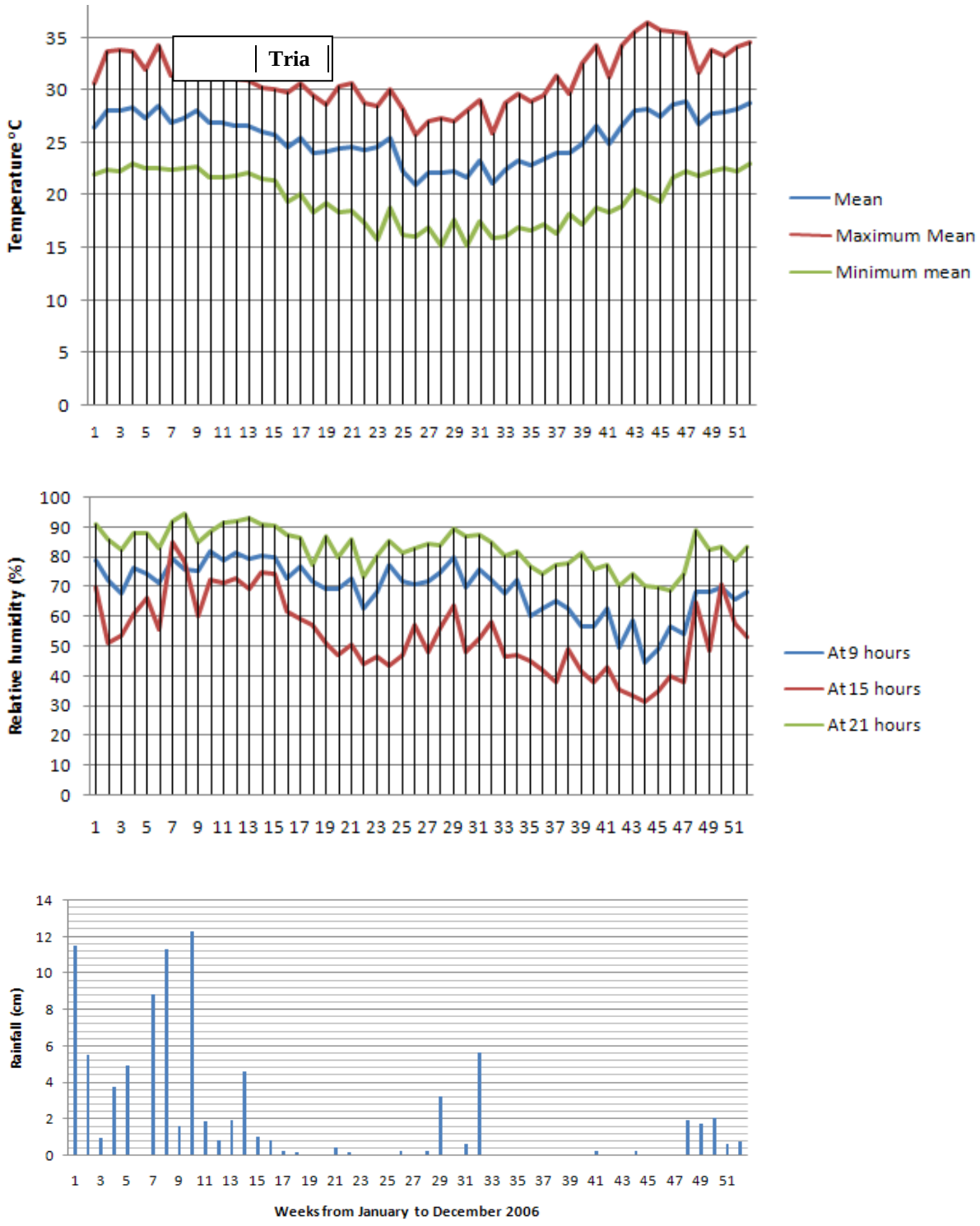


Figure 5.1: Temperature, air relative humidity and rainfall weekly means at Nassuruma in 2006.

Table 5.1: *Colletotrichum gloeosporioides* naturally inoculated seedlings: Number of leaves, disease incidence and severity means per assessment date at Nassuruma, Mozambique

Statistic & Days of exposure	Assessed variate Seedling Pheno-stage	Number of leaves	Disease incidence (%)	Disease severity (%)
1	Young	5.70[a]	0.00	0.00
	Intermediate	8.40b	0.00	0.00
	Old	14.50c	0.00	0.00
SEM		0.258		
Probability		<0.001		
LSD(%)		1.12		
CV(%)		6.1		
2	Young	5.70[a]	0.00	0.00
	Intermediate	8.45b	1.67	0.016
	Old	14.45c	4.17	0.040
SEM		0.229	1.226	0.01178
Probability		<0.001	0.092	0.092
LSD(%)		0.99	n/a	n/a
CV(%)		5.4	141.0	141.1
3	Young	6.20[a]	0.8[a]	0.008[a]
	Intermediate	8.50b	18.3ab	0.182b
	Old	14.70c	23.3b	0.232b
SEM		0.295	4.28	0.0433
Probability		<0.001	0.007	0.008
LSD(%)		1.28	18.47	0.1868
CV(%)		6.7	67.5	68.8
4	Young	6.40[a]	6.7[a]	0.066[a]
	Intermediate	8.45b	28.3b	0.28b
	Old	14.45c	36.7b	0.368b
SEM		0.279	3.35	0.0339
Probability		<0.001	<0.001	<0.001
LSD(%)		1.21	14.47	0.1464
CV(%)		6.4	31.4	31.7
5	Young	6.65[a]	21.7	0.216
	Intermediate	8.60b	44.2	0.442
	Old	14.45c	46.7	0.466
SEM		0.346	6.29	0.0627
Probability		<0.001	0.030	0.029
LSD(%)		1.50	n/a	n/a
CV(%)		7.8	37.5	37.4
6	Young	6.70[a]	35.8	0.402
	Intermediate	8.45b	50.0	0.508
	Old	14.55c	55.0	0.566
SEM		0.360	5.49	0.0579
Probability		<0.001	0.073	0.170
LSD(%)		1.56	n/a	n/a
CV(%)		8.1	26.1	26.3
7	Young	6.80[a]	43.3	0.516
	Intermediate	8.60b	55.8	0.564
	Old	14.15b	66.7	0.808
SEM		0.501	6.24	0.0730
Probability		<0.001	0.063	0.033
LSD(%)		2.17	n/a	n/a

CV(%)		11.4	25.2	25.9
8	Young	6.30[a]	43.3	0.574
	Intermediate	8.15[a]	55.0	0.556
	Old	14.60b	67.5	0.716
SEM		0.471	6.38	0.0747
Probability		<0.001	0.060	0.290
LSD(%)		2.04	n/a	n/a
CV(%)		10.9	25.8	27.2
9	Young	7.20[a]	56.7	0.710
	Intermediate	8.45[a]	67.5	0.692
	Old	14.20b	77.5	1.000
SEM		0.578	6.44	0.0778
Probability		<0.001	0.114	0.027
LSD(%)		2.50	n/a	n/a
CV(%)		13.0	21.4	21.7
10	Young	7.40[a]	62.5	0.808
	Intermediate	8.75[a]	76.7	0.794
	Old	14.35b	75.8	0.908
SEM		0.406	4.99	0.0737
Probability		<0.001	0.120	0.510
LSD(%)		1.76	n/a	n/a
CV(%)		8.9	15.6	19.7
11	Young	7.25[a]	71.7	1.032
	Intermediate	8.70[a]	82.5	0.850
	Old	14.35b	86.7	1.134
SEM		0.494	3.25	0.0943
Probability		<0.001	0.018	0.140
LSD(%)		2.13	n/a	n/a
CV(%)		10.9	9.0	21.0
12	Young	7.65[a]	75.0a	1.160
	Intermediate	8.80[a]	87.5b	0.0918
	Old	14.30b	95.0b	1.268
SEM		0.404	2.68	0.1258
Probability		<0.001	<0.001	0.174
LSD(%)		1.75	11.57	n/a
CV(%)		8.8	7.0	25.2

SEM = Standard error; LSD = Least significant difference and C V = Coefficient of variance.

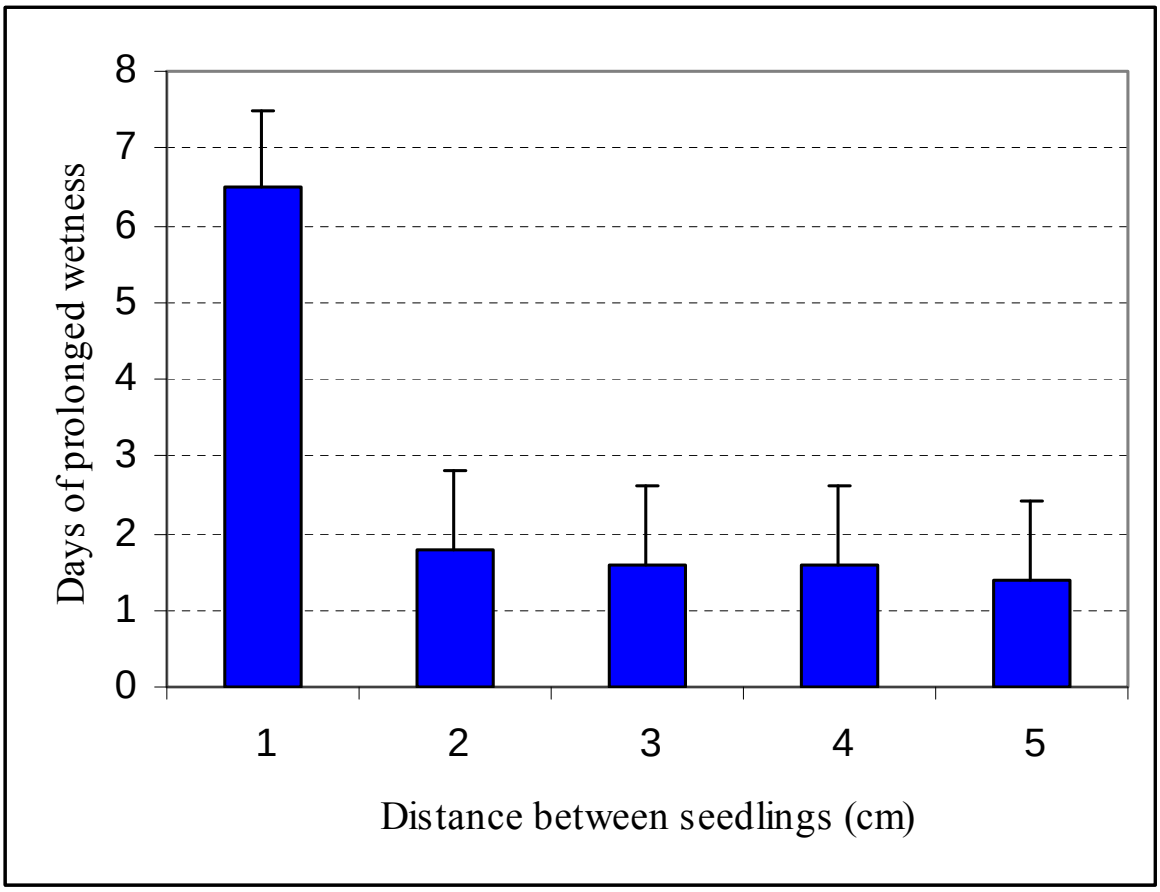


Figure 5.2: Effect of cashew seedling density on the number of days of wetness at Nassuruma, 2007 out of 13 days of mist. Vertical lines on top of bars represent standard error.

CHAPTER VI: FREQUENCY OF HEXACONAZOLE APPLICATIONS FOR CASHEW ANTHRACNOSE AND POWDERY MILDEW CONTROL IN MOZAMBIQUE

6.1. Abstract

Weekly applications of the fungicide 5% hexaconazole SC starting at different cashew development phenological stages (flushing, flowering and fruiting) were investigated as a strategy for simultaneous control of anthracnose and powdery mildew diseases on cashew. The trial was conducted during the 2006 and 2007 crop seasons and followed a completely randomized block design with four replicates and four treatments. Incidence of anthracnose on leaves and powdery mildew severity on panicles were assessed. The economic implication of high frequency application of hexaconazole in the current context of fungicide use on cashew in Mozambique was evaluated. A maximum of 13 applications starting from flushing successfully prevented the development of powdery mildew and significantly reduced the incidence of leaf anthracnose. The best economic profit was obtained with moderate bio-efficacy after 10 applications of hexaconazole for cashew trees with potential yield of at least 25 kilograms per tree per season.

6.2. Introduction

Cashew anthracnose is caused by the fungus *Glomerella cingulata* (Ston.) Spauld. & Schrenk, the teleomorph of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Ohler, 1979; Milheiro and Evaristo, 1994). It is unknown when the disease was introduced into Mozambique but the first official record was by De Carvalho and Mendes (1958). The economic relevance of the disease was highlighted after a nationwide survey was undertaken by Dhindsa and Monjana (1984). In their study, they recommended chemical use for the effective management of the disease.

In Brazil, where yield losses up to 50% have been reported, fungicides such as copper oxychloride, copper hydroxide, zinc + manganese carbamate, captafol, benomyl, dithianon, anilazine, bitertanol, triadimenol and triforine, are used to control the disease (Freire *et al*., 2002). However, the choice of fungicides depends on various factors such as biological

efficiency, crop sensitivity (Freire and Cardoso, 1995), economic feasibility (Freire *et al*., 2002), environmental aspects (Stirling *et al*., 1999), previous exposure to a certain fungicide or group (Karadimos, 2005) and local regulations for registration (Arauz, 2000).

In Mozambique, only copper oxychloride is used to control cashew anthracnose. Other fungicides such as 5% trifloxystrobin WG, 25% difeconazole EC and 25% azoxystrobin EC were proven also to be effective (Uaciquete and Lyannaz, 2003; Uaciquete and Lyannaz, 2004). However, these fungicides have not been widely adopted due to similar constraints as described in Brazil, i.e. extensive area planted but low productivity and the high cost of these fungicides (Freire *et al*., 2002). In Mozambique, the cost is increased because other and timely asynchronized series of fungicide applications are required for powdery mildew (*Oidium anacardii* Noack) control.

In Mozambique cashew fungicides are 100% subsidized by the government, including transportation up to farm gate. Farmers pay only for the spraying service. Because the incidence of powdery mildew (PMD) is higher in the Southern as compared to the Northern Mozambique, the recommended frequency of fungicide application has been four and three times respectively.

Although most commonly reported (Ohler, 1979; Intini, 1987; Freire and Cardoso, 1995; Cardoso *et al*., 1999; Ikisan, 2000; Freire and Cardoso, 2002; Oliveira, 2002; Freire *et al*., 2002; Cardoso and Freire, 2003), copper fungicides are very old, have a residual build up in soils and were found to be detrimental to avocado phylloplane microorganisms with natural suppressive effect on *Colletotrichum gloeosporioides* (Stirling *et al*., 1999). Alternatively, hexaconazole is widely used in cashew powdery mildew control but it has shown no significant reduction on anthracnose severity. The current study was conducted to determine the effectiveness of 5% hexaconazole SC against both cashew anthracnose and powdery mildew through changes in timing and frequency of applications and thus minimize the cost of applying two chemicals at different stages.

6.3. Material and methods

6.3.1. Location, plants and experimental design

The present experiment was conducted during 2006 and 2007 cropping seasons in a private farm in Rapale District (latitude, 14° 00' South and longitude, 36° 27' East), from May/June till December each year. The farm trees were seven years old, grafted and planted in a 10 x 10 m spacing. Experimental trees were chosen on the basis of uniformity in terms of height (6-10 m tall) and canopy shape (roughly hemispherical canopies) and absence of interlocking branches with adjacent trees (Smith *et al*., 1995).

A randomized block design was adopted for the trial (Petersen, 1994). Four blocks with four treatments were selected for this trial. Treatments represented different dates at which field sprays of 5% hexaconazole SC (Volcano Richter, VOLCANO Agroscience (Pty) Ltd., Durban, RSA) were initiated. The first treatment aimed at protecting the plants from the red leaf stage onwards; the second treatment was aimed at protecting the plant from panicle emission stage onwards and the third, from fruiting and then a fourth treatment that consisted of un-sprayed trees served as control (Table 6.1). Experimental plots consisted of five plants and the plot was separated from another by at least one row in each direction. The applied dose was 0.5 ml of active ingredient per tree (Ngovene and Cumbi, 2003).

6.3.2. Spraying procedure

Spraying was carried out using a motorized knapsack mist blower (SOLO 423) operated at release level 3 by a trained person. Detailed procedures such as speed of the operator, wind challenge and canopy coverage have been previously described (Masawe *et al*., 1997; Smith *et al*., 1997; Sijaona and Mansfield, 2001) and they were observed. The insecticide karate 5% EC (Lambda cyhalothrin; Syngenta Crop Protection, AG, Basel, Switzerland) was applied once at emergence of panicles, for the control of aphids and tea mosquito pests at the rate of 0.25 ml of active ingredient per tree (Boma *et al*., 1995).

6.3.3. Data collection

Both anthracnose leaf incidence and powdery mildew severity on flowers were evaluated. Before the initiation of generative growth or bud burst stage (Conticini, 1982, Appendix 2; Appendix 3), each cashew tree canopy was divided into two sides (shady and sunny). From each side of the tree, five shoots were sampled and tagged as described previously (Sijaona and Mansfield, 2001).

Anthracnose severity was scored weekly (Table 6.2), in a maximum of ten young leaves per shoot. A scale (0-6) developed by Nathaniels (1996) for powdery mildew coverage on leaf surface was adopted. The area covered by powdery mildew in the scale, corresponds well with the necroticised area in the case of anthracnose infection (Appendix 5).

Powdery mildew severity assessment was made on panicles following a 0-6 standard key that estimates the percentage of flowers and flower buds affected (Nathaniels, 1996; Topper *et al*., 1998; Sijaona and Mansfield, 2001; Appendix 4). Three to four observations were made in order to cover the peak of disease intensity as observed from the untreated plots.

6.3.4. Statistical analysis

For both anthracnose and powdery mildew, individual plant score means for shoots and panicles were manually calculated in the field forms as detailed before (Nathaniels, 1996; Masawe *et al*., 1997 and Sijaona and Mansfield, 2001). Then, plant means, over the years, were used to run the statistical analysis for both anthracnose incidence and powdery mildew severity. Analysis of variance (ANOVA) was used to determine differences between the treatment effects on means. The data was acceptably normally distributed with heterogeneous treatment variances. Means were therefore separated using Fisher's protected t-test least significant difference (LSD) at 1% level of significance (Snedecor and Cochran, 1980). Data was analyzed using the statistical program GenStat (2003).

6.4. Results

6.4.1. Effect of high frequency application of hexaconozole on disease incidence and severity

Fungicide applications aimed at protecting emerging young leaves from flushing stage began on June (Table 6.1) and yielded a maximum total number of 13 sprays. Young leaf emergence was observed one week earlier in 2007/2008 crop season compared to the previous year. Flowering consistently started three weeks after flashing and thus hexaconazole applications starting at this stage were 10. Hexaconazole applications to protect fruits were 2 and 3 in 2006 and 2007 respectively (Table 6.1).

Regardless of when the sprayings started, powdery mildew severity mean scores for two consecutive seasons were significantly lower on hexaconazole treated trees compared to untreated controls (Table 6.2). In fact, no powdery mildew symptoms developed on panicles when weekly applications of hexaconazole started at flushing stage. However, disease symptoms were observed with significant increase when applications were initiated at flowering and fruiting (Table 6.2).

On hexaconazole sprayed trees, starting in June, at the flushing stage, anthracnose mean incidence on emerging leaves was 1.75%. This represents five a times reduction when compared to 9% mean incidence on untreated trees (Table 6.2). However, when applications were initiated in July, aiming to protect flowers or fruits, no statistical differences with untreated trees were detected (Table 6.2). Similarly, highly significant reduction of anthracnose severity on leaves was observed when hexaconazole was applied right from the flushing stage: 0.08 against 1.36% on untreated plants. .

Anthracnose incidence on fruits was significantly reduced by weekly application of hexaconazole compared to untreated trees regardless of when the spraying started (Table 6.2). However, no significant differences were detected between stages of initiating applications.

6.4.2. Financial impact of hexaconazole high frequency application

In Mozambique, two to three applications of copper oxychloride at two weeks interval are required to suppress anthracnose epidemics. However, for powdery mildew, depending on geographic region and climate, three to five fungicide sprayings per cropping season may be necessary. Our results indicated that starting hexaconazole applications at flowering prevented PMD development and significantly reduced leaf and fruit anthracnose incidence (Table 6.2). Thus, a total of ten weekly applications of hexaconazole from flowering were necessary (Table 6.1). The profit, on the basis of this frequency and only nut yield, is 4.14 kg/tree, equivalent to 29 Mt/tree (Table 6.3). This profit is 46.8 and 43.3% of that under no anthracnose epidemic circumstances in the Southern and Northern regions respectively. Maximum significant biological effectiveness on both diseases was obtained after 13 weekly sprayings of hexaconazole (Table 6.1) and the profit margin was further eroded to only 10.32 Mt/tree (Table 6.3).

6.5. Discussion

An effective control of two diseases using a single fungicide spraying plan is the desirable strategy today in Mozambique. In this trial, leaf and fruit anthracnose spread (incidence) on cashew young shoots at Rapale farm during two crop seasons, was significantly reduced by ten weekly applications of hexaconazole 5% SC starting at flowering as compared to untreated plots. The same fungicide application plan was also highly effective against powdery mildew severity. Simultaneous management of anthracnose and powdery mildew on mango has been reported in a 25 times application plan of fungicides per season (Redondo, 2000; Arauz, 2000). When to initiate the spraying is a critical aspect. In Brazil, using a 0 to 4% leaf area damage scale and chemical action is recommended when 2% of the assessed leaf area has developed necrosis (Cardoso *et al*., 2002). Adopting an action point of two percent disease severity implies starting applications at flowering. In our experiment, this coincided with the most profitable timing of applications. Therefore, confirming the findings of Cardoso *et al*. (2002).

In Mozambique, not scouting is common, due to logistics, high rate of disease spread (Topper *et al*.,1998), endemic nature of the disease (Nathaniels, 1996), phenologically heterogeneous plantations (Ferrão, 1995) and time and labour consuming for scouting procedure. Consequently, spraying service providers instead, start sprayings following the plant

phenology (flowering phase) rather than scouting individual plants. However the use of a measurable action point avoids un-necessary sprays with all ecological concerns and development of resistance by the pathogen as referred by Redondo, (2000).

Field evaluation, including economic aspects of control of multiple pathogens on cashew by hexaconazole has been recommended, following an *in vitro* demonstration of high spectrum of activity on cashew pathogens (CHAPTER IV). Hence the product has revealed its effectiveness in the field preventing significantly both anthracnose spread and severity and (powdery mildew) severity. In current study, a conservative profit of 29 Mt/tree after ten applications and only 10.32 Mt/tree at maximum bio-efficacy, after 13 sprays. This profit is conservative because, as in many cases in East Africa, the value of cashew apple is officially neglected despite its traditional high value. In addition, cashew nut price varies from 6 to 12 Mt in a season and yield may also reach as high as 25 Kg/tree.

In cashew anthracnose disease epidemics, high relationships between incidence and severity on leaves have been demonstrated (CHAPTER VII). But, high association between cashew anthracnose incidence on young leaves and subsequent incidence on young fruits to our knowledge is hereby reported for the first time.

Insecticide was applied once but not included in economic evaluation. It was used as a mixture with fungicide when mosquito bug and aphids infestation was noticed. The cost of spraying insecticide was considered only for the fungicide. Occurrence of these pests on cashew is well documented in Tanzania and India (Intini, 1987). In fact, *Helopeltis* infestation has been reported as facilitator for *Colletotrichum gloeosporioides* penetration (Ohler, 1979) and consequently an integrated (Fungicide + insecticide) approach recommended (Boma *et al*., 1995).

Effective and economic control of both anthracnose and powdery mildew diseases was achieved by spraying at least ten times hexaconazole, starting at flushing phenological phase of the crop and therefore the fungicide application plan is hereby recommended.

References

Arauz, L.F. 2000. Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease 84:600-611*.

Boma, F., Topper, C.P. & Madson, A. 1995. Cashew Improvement Programme. Annual cashew research report 1994-1995. ODA cashew research project. Agricultural Research Institute, Naliendele.

Cardoso J.E., Cavalcanti, J.J.V., Cavalcante, M. de J.B., Aragão, M. do L. & Filipe, E.M. 1999. Genetic resistance of dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mold, and angular leaf spot. *Crop Protection 18: 23-27*.

Cardoso, J.E., dos Santos, A.A., Freire, F.C.O., Viana, F.M.P., Vidal, J.C., Oliveira, J.N. & Uchoa, C.N. 2002. Monitorimento de doenças na cultura do cajueiro. Embrapa, Agroindustria Tropical, Fortealeza-CE.

Cardoso, J.E., dos Santos, A.A., Rossetti, A.G. & Vidal, J.C. 2004. Relationship between Incidence and severity of cashew gummosis in semiarid north-eastern Brazil. *Plant Pathology 53: 363-367*.

Conticini, L. 1982. Guida fenologica dell'anacardio (*Anarcadium occidentale* L.) *Revista di Agricoltra Subtropicale e Tropicale 76:221-236*.

Carvalho, T. & Mendes, O. 1958. Doenças de Plantas em Moçambique. Departamento de Sanidade Vegetal, Instituto de Investigação Agronómica, Moçambique. 84 p.

Dhindsa, P.P. & Monjana, M. 1984. Index of plant diseases and associated organisms of Mozambique. *Tropical Pest Management 30: 407-429*.

Ferrão, J.E.M. 1995. O cajueiro (*Anacardium occidentale* L.). Instituto de Investigação Cientifica Tropical, Lisboa. 298 p.

Freire, F.C. & Cardoso, J.E. 1995. Doenças do cajueiro. Pages 250-267. In Cajucultura. Modernas técnicas de produção. De Araujo, J.P.P. & Da Silva, V.V. (eds). EMBRAPA-CNPAT, Fortaleza.

Freire, F.C.O., Cardoso, J.E., Dos Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21: 489-494*.

Intini, M. 1987. Phytopathological aspects of cashew (*Anacardium occidentale* L.) in Tanzania. *International Journal of Tropical Plant Diseases 5:115-130*.

Intini, M. & Sijaona, M.E.R. 1983. Little known diseases of cashew (*Anacardium occidentale* L.) in Tanzania. *Revista di Agricoltura Subtropicale e Tropicale 77:421-429*.

Karadimos, D.A., Karaoglanidis, G.S. & Tzavella-Klonari, K. 2005. Biological activity and physical modes of action of Qo inhibitor fungicides trifloxystrobin and pyraclostrobin against Cercospora beticola. *Crop Protection 24:23-29.*

Masawe, P.A.L., Cundal, E.P. & Caligari, P.D.S. 1997. Powdery mildew (*Oidium anacardii*) onset and development on flowering panicles of cashew clones (*Anacardium occidentale* L.) as a measure of clone resistance. *Tropical Agriculture 79:229-234*.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do Cajueiro. Cultivar. Associação de Técnicos de Culturas Tropicais, Porto. 204 p.

Ngovene, V. & Cumbi, J. 2003. Avaliação da eficacia de fungicidas no controlo do oidio do cajueiro (*Oidium anacardii* Noack). Pages 32-36 *in:* Relatório Anual 2002/2003. Programa Nacional de Investigação do caju. Instituto Nacional de Investigação Agronomica, Maputo.

Ohler, J.G. 1979. Cashew. Koninklijk Instituut voor de Tropen, Amsterdam, Netherland. 260 p.

Payne, R.W. 2003. GenStat® for Windows® (7th Edition). VSN International. Hemel Hempstead. 56 p.

Petersen, R.G. 1994. Agricultural field experiments. Design and analysis. Marcel Dekker, Inc., New York. 409 p.

Redondo, A.R.P. 2000. Nueva estrategia para el manejo en campo de la antracnosis (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.) del mango (*Mangifera indica* L.). *Fitopatologia Colombiana 24:21-28*.

Sijaona, M.E.R. & Mansfield, J.W. 2001. Variation in the response of cashew genotypes to the targeted application of fungicide to flower panicles for control of powdery mildew disease. *Plant Pathology 50:224-248*.

Silinto, B.F. 2005. Influência de alguns factores ambientais no desenvolvimento da anthracnose nas folhas do cajueiro (*Anacardium occidentale* L.). Trabalho de Licenciatura. Faculdade de Ciências. Universidade Eduardo Mondlane, Maputo.

Smith, D.N., King, W.J., Topper, C.P., Mhando, H. & Cooper, J.F. 1997. Studies on spray deposition on cashew trees in Tanzania with reference to uses of fungicides to control *Oidium anacardii*. *Crop Protection 16:313-322*.

Smith, D.N., King, W.J., Topper, C.P., Boma, F. & Cooper, J.F. 1995. Alternative techniques for application of sulphur dust to cashew trees for the control of powdery mildew caused by the fungus *Oidium anacardii* in Tanzania. *Crop Protection 14:555-560*.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical methods (7th edition). Ames: Iowa State University Press. 807 p.

Stirling, A.M., Pegg, K.G., Hayward, A.C. & Stirling, G.R. 1999. Effect of copper fungicides on *Colletotrichum gloeosporioides* and other micro organisms on avocado leaves and fruit. *Australian Journal of Agricultural Research 50:1459-1468*.

Topper, C.P., Boma, F. & Anthony, J. 1998. Screening trials for the evaluation of fungicides for the control of powdery mildew (*Oidium anacardii* Noack) on cashew in Tanzania. B. Using inflorescences on young trees. Pages 291-294 *in* Proceedings of the International cashew and Coconut Conference, 17-21 February, 1997. Dar es Salaam. Topper, C.P., Caligari, P.D.S.,

Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe P.A.L & Mpunami, A.A. (eds), BioHybrids International Ltd. Reading, UK.

Topper, C.P., Boma, F. & Anthony, J. 1998. Screening trials for the evaluation of fungicides for the control of powdery mildew (*Oidium anacardii* Noack) on cashew in Tanzania. A. Using leaves of seedling plants. Pages 286-290 *in*: Proceedings of the International cashew and Coconut Conference, 17-21 February, 1997. Dar es Salaam. Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe P.A.L & Mpunami, A.A. (eds), BioHybrids International Ltd. Reading, UK.

Topper, C.P., Boma, F. & Mhando, H. 1997. Evaluation of fungicides for the control of powdery mildew (*Oidium anacardii* Noack) on cashew in Tanzania. A. Fungicide strategy development trials. Pages 254-259 *in*: Proceedings of the International cashew and Coconut Conference, 17-21 February, 1997. Dar es Salaam. Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe P.A.L & Mpunami, A.A. (eds), BioHybrids International Ltd. Reading, UK.

Uaciquete, A. & Lyannaz, J.P., 2003. Avaliação preliminar de fungicidas para o controlo de anthracnose (*Colletotrichum gloesporioides* Penz.) no cajueiro (*Anacardium occidentale* L.) em Moçambique. Pages 37-44, *in*: Relatório Anual 2002/2003. Programa Nacional de Investigação do caju. Instituto Nacional de Investigação Agronomica, Maputo.

Uaciquete, A. & Lyannaz, J.P. 2004. Avaliação de fungicidas para o controlo de anthracnose (*Colletotrichum gloesporioides* Penz.) no cajueiro (*Anacardium occidentale* L.) em Moçambique. Pages 40-45, *in*: Relatório Anual 2003/2004. Programa Nacional de Investigação do caju. Instituto Nacional de Investigação Agronomica, Maputo.

Table 6.1: Date of application of hexaconazole per treatment, during 2006 and 2007 cashew crop seasons, Rapale District, Mozambique

Date of application 2006		**June 16**	**June 23**	**June 30**	**July 7**	**July 18**	**July 25**	**August 1**	**August 8**	**August 15**	**August 23**	**August 29**	**September 7**	**September 15**
Treatment	Flushing	*	*	*	*	*	*	*	*	*	*	*	*	*
	Flowering				*	*	*	*	*	*	*	*	*	*
	Fruiting												*	*
	Un-treated control													
Date of application 2007		June 10	June 17	June 25	July 2	July 9	July 16	July 23	July 30	August 1	August 8	August 16	August 23	September 1
Treatment	Flushing	*	*	*	*	*	*	*	*	*	*	*	*	*
	Flowering				*	*	*	*	*	*	*	*	*	*
	Fruiting											*	*	*
	Un-treated control													

Table 6.2: Effect of weekly applications of hexaconazole 5% on cashew anthracnose and powdery mildew, during 2006 and 2007 crop seasons in a private farm, Rapale district, Mozambique

Crop season	Disease	Anthracnose			Powdery mildew
	First application at	Severity (%)	Incidence (%)		Severity (%)
		on leaves	on leaves	on fruits	on panicles
2006	Flashing	0.08(0.75)a	1.75(0.73)a	0.15(0.72)a	0.00(0.70)a
	Flowering	0.69(0.97)b	5.66(0.80)b	0.12(0.73)ab	2.78(1.61)b
	Fruiting	0.88(1.01)bc	6.30(0.79)b	1.08(0.80)b	12.30(4.70)c
	Control (un-treated)	1.36(1.13)c	9.00(0.81)b	4.39(1.06)c	24.35(3.30)d
	C V (%)	00(49.02)	00(11.56)	00(118.6)	40.56
	Tukey's prob.(alpha=0.05)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)
2007	Flashing	0.09(0.70)a	2.75(0.45)a	0.10(0.12)a	1.01(0.30)a
	Flowering	0.70(1.00)b	3.66(0.60)b	0.24(0.13)ab	3.08(0.71)ab
	Fruiting	0.60(1.01)b	8.30(0.79)b	0.98(0.60)b	10.10(2.70)c
	Control (un-treated)	2.36(1.13)c	10.00(0.21)b	5.30(1.00)c	20.12(2.30)d
	C V (%)	00(39.45)	00(12.16)	00(120.6)	39.46
	Tukey's prob.(alpha=0.05)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)

Numbers in ( ) are results of square root data transformation; C V (%) = Coefficient of variance; Means followed by the same letter in a column, are not statistically different

Table 6.3: Economic impact of increased frequency of application of hexaconazole 5% SC for the control of anthracnose and powdery mildew on cashew in Mozambique

	Desease& Region	Price/Application (Mt). A	Frequency of applications. B	Price/tree/season (Mt). A*B	Yield/tree/season (kg). C	Price/Kg(Mt). D	Total gain/tree/season (Mt). C*D	Profit/tree/season. C*D-A*B
Recommended	PMD-SM	5.5	4	22	6	13	78	56
	PMD-NM	5.7	3	17.1	6	13	78	60.9
Suggested by this study, at minimum yield/tree	PMD+A-SM	5.5	10	55	6	13	78	23
	PMD+A-SM	5.7	13	71.5	6	13	78	6.5
	PMD+A-NM	5.5	10	57	6	13	78	21
	PMD+A-NM	5.7	13	74.1	6	13	78	3.9
Suggested by this study, at average yield/tree	PMD+A-SM	5.5	10	55	12	13	156	101
	PMD+A-SM	5.7	13	71.5	12	13	156	8.5
	PMD+A-NM	5.5	10	57	12	13	156	99
	PMD+A-NM	5.7	13	74.1	12	13	156	81.9
Suggested by this study, at best yield/tree	PMD+A-SM	5.5	10	55	25	13	325	270
	PMD+A-SM	5.7	13	71.5	25	13	325	253.
	PMD+A-NM	5.5	10	57	25	13	325	268
	PMD+A-NM	5.7	13	74.1	25	13	325	250.9

PMD = Powdery mildew disease; A= Anthracnose disease; SM = Southern Mozambique and NM = Northern Mozambique.

CHAPTER VII: RELATIONSHIP BETWEEN INCIDENCE AND SEVERITY OF CASHEW ANTHRACNOSE (*COLLETOTRICHUM GLOEOSPORIOIDES*) IN MOZAMBIQUE

7.1. Abstract

Anthracnose of cashew was studied on various genotypes and locations in Mozambique. The anthracnose causal agent was identified using polymerase chain reaction technique. The relationships between incidence and severity of anthracnose on cashew genotypes were statistically analyzed by regression. Anthracnose leaf incidence, which is practically easy to evaluate, was consistently associated with leaf severity and their relationships can be estimated using the restricted exponential function across locations, crop seasons, genotypes and fungicide trials. Pooled data were used to estimate initial incidence of 1.43% with percentage variance accounting for 83.2 and standard error of 8.32. By computing incidence data into summary equation, changes of 1, 5, 10, 40 and 60%, resulted in changes of severity estimates of 0.01, 0.05, 0.10, 0.50 and 1% respectively. Estimate for maximum disease incidence was 80% when the severity reached only 5%. When the severity approached a maximum of 25% leaf detachment was observed. The use of incidence data for epidemic comparisons, genotype and fungicide evaluation in cashew orchards is recommended. Anthracnose incidence on leaves however, could not predict incidence on nuts.[2]

[2] Article published in *Crop Protection 49 (2013) 66-72.*

7.2. Introduction

Cashew anthracnose is caused by *Colletotrichum gloeosporioides*, and its symptoms manifest in both leaves and young nuts (Freire *et al*., 2002). Severe damage on adult plants results in defoliation during shoot development, death of inflorescences and later necrosis and falling of immature nuts (Freire and Cardoso, 2003). In Mozambique, cashew anthracnose symptoms have been described (Carvalho and Mendes, 1958). Since *Colletotrichum* species are no longer reliably separated by cultural and morphological characteristics (Muniz *et al*., 1998; Ivey *et al*., 2004; Whitelaw-Weckert, *et al*., 2007), we hypothesized that other species of *Colletotrichum* may be involved in the cashew anthracnose complex.

Once established the identity of the pathogen, damage assessment can be done for fungicide or germplasm screening (Da Matta and Lellis, 1973; Cardoso *et al*., 1999) or epidemiological investigations (McRoberts *et al*., 2003; Cardoso *et al*., 2004). In any of the above approaches, terminology such as disease incidence, disease severity, disease density and others are commonly used to measure the disease. Relative advantages and practical applications of their relationships have been discussed (McRoberts *et al*., 2003). Nevertheless, practical limitations resulting from inconsistency of the relationships across locations, stage of the epidemic, host genotype and crop cycle have been found (James and Shih, 1973; Rouse *et al*., 1981; Chuang and Jeger, 1987). Many other authors (Silva-Acuna *et al*., 1999) have however found simple, consistent and useful relationships in different pathosystems. Tedious and time consuming work associated with severity measurement has been replaced by the easily measured incidence (Silva-Acuna *et al*., 1999; Cardoso *et al*., 2004).

In this study we aimed at (i) establishing the identity of the causal agent of cashew anthracnose using PCR technique, (ii) using standardized visual keys to describe the relationship between incidence and severity, (iii) comparatively characterizing the development of cashew anthracnose epidemics across seasons, genotypes and locations and (vi) exploring the possibility of developing a predictive model for incidence on nuts based on incidence or severity on leaves produced before the setting of nuts.

7.3. Material and methods

7.3.1. Pathogen identification

Anthracnose symptomatic samples were collected from all the trial sites and pathogen isolations made on potato dextrose agar (PDA). Fungal mycelia were then harvested from PDA cultures in laminar hood and total genomic DNA extracted using the DNeasy Plant Mini Kit (Qiagen Inc.) procedures. Mycelium of *C. gloeosporioides* from mango, was used as positive control. For PCR amplification, species-specific primers from the ITS1 region of the ribosomal DNA gene CgInt2 (5'-GGC-GCCGGCCCCGTCACGGGGG-3') and CgInt (5'-GGCCTCCCGCCTCCGGGCGG-3') for *C. acutatum* and *C. gloeosporioides* respectively, were individually coupled with the universal and conserved primer ITS4 (Ivey *et al*., 2004; Whitelaw-Weckert *et al*., 2007). Amplification reactions were performed in an Eppendorf Master Thermocycler (Merck Chemicals Pty Ltd, South Africa). Each reaction mix (µl) contained: 0,5 µl DNA, 0.5 µl of 2.5 mM each dNTP, 1.25 µl of 50 mM $MgCl_2$, 1x NH_4 reaction buffer, 0.3 µl of each 1x diluted primer (24,20 nmol CgInt2; 25,30 nmol CgInt and 15 mg/l ITS4), 0.25 µl of Taq DNA polymerase. Amplification cycles were as described by Ivey *et al*. (2004). PCR products (0.5 µl) were separated by horizontal gel electrophoresis in a Maxicell EC360M electrophoretic gel system (Electrophoretic gel system, E-C Apparatus Corporation) coupled to a 250/2.5 voltmeter model (Bio-Rad, South Africa). One percent agarose gels were immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0) at 100 V for 60 min. The gels contained 10 mg/ml ethidium bromide as stain. The DNA bands were visualized under UV light and photographed with the aid of Vilber Lourmat photosytem (Marne la Vallee, France).

Cashew orchards were located in four sites of Northern Mozambique namely, Nassuruma, Rapale, Mocuba and Pebane (Table 7.1). The plants were rain fed and cropping practices consisted of weeding and application of fungicides against powdery mildew. Fungicides used were: Volcano Richter (hexaconazole SC 5%, Volcano AgroScience (Pty) Ltd) and Voltriad (Triadimenol SC 5%, Volcano AgroScience (Pty)) both at a rate of 10ml/L/tree of formulated product, three times a season at 21-day intervals (Sijaona and Mansfield, 2001). At Rapale, data were collected from a fungicide trial with weekly applications of hexaconazole.

7.3.2. Disease field assessment

At the beginning of each crop season, from both north and south sides of individual trees, five shoots were tagged with a sisal cord of approximately 25 cm (Sijaona and Mansfield, 2001).

The disease was assessed on a maximum of ten new leaves per time per shoot and for two consecutive crop seasons 2006 and 2007. Assessments began in May/June and ended in September according to the development and maturation of shoots. Incidence and severity were evaluated simultaneously based on the standardized leaf damage scale (Appendix 5). In this study, severity described the percentage of necroticised leaf area while incidence reflected the percentage of diseased leaves out of the total evaluated (McRoberts *et al*., 2003; Cardoso *et al*., 2004). Later in each crop season, anthracnose incidence on the nuts was also assessed as percentage of symptomatic immature nuts/panicle/plant. Disease scores were initially processed to return plant mean scores as detailed by Masawe *et al*., (1997).

Regression analysis of incidence and severity from un-transformed data were performed using GenStat (2003) package for windows. Variables means over date and treatment were computed to fit an exponential function (Snedecor and Cochran, 1980; Cornell and Berger, 1987). Incidence was the response variant and severity the explanatory (McRoberts *et al*., 2003; Cardoso *et al*., 2004). Furthermore, leaf severity and incidence were used as explanatory to the incidence on nuts.

Daily rainfall data were obtained from the closest district directorate of agriculture of each site. Weekly sums were computed and graphically represented for each location.

7.4. Results

7.4.1. Pathogen identification

The species specific primer CgInt coupled with the ITS4 primer successfully amplified the same size fragment from genomic DNA of all forty *Colletotrichum* isolates as the positive reference *C. gloeosporioides* isolate from mango. No additional band was observed closer to the reference therefore no PCR-amplified product for primer CgInt2 was detected. Negative controls had no amplification product.

7.4.2. Disease field assessment

The relationship between incidence and severity on the cashew leaf anthracnose pathosystem was consistently best characterized by the restricted exponential function ($P<0.001$): $I = b\ (1-e^{-as})$ across locations, crop seasons, cashew genotype and fungicide trials (Table 7.2). In this function, I stands for incidence, S for severity, *b* for estimated maximum incidence (emi) and *a* stands for estimated initial disease incidence (eii).

The potential for epidemics in each location or crop season was expressed by the emi value (asymptote of the restricted exponential curve) noting however that the explicit maximum is 100% (McRoberts *et al*., 2003). Thus, at Nassuruma germplasm screening trial, for the 2007 crop season the emi value was 100.0% against 68.56% for 2006. Therefore year 2006 was less conducive to disease spread than the following crop season (Fig. 7.1).

Estimated initial incidence (eii) in the restricted exponential function (Table 7.2) expresses the abundance of inoculum or minimum aggregation of the pathogen that is not visually recordable (McRoberts *et al*., 2003). In the cashew anthracnose pathosystem, such inoculum may derive from nearby infected plants and mummified fruits. More predominantly inoculum may come from leaves of previous vegetative growth that develops before the reproductive growth within a year. Thus, at Nassuruma, the eii value was higher (8.74%) in the 2006 crop season than 2007 (3.00%) (Table 7.2).

In contrast, at Mocuba, the year 2006 crop season showed a relatively higher emi value compared to year 2007 for both dwarf and common types (Fig. 7.2 A&B, D&E). At Mocuba and Pebane the eii values of both dwarf and common cashew type trials for the crop season 2006 were lower compared to year 2007 (Table 7.1). This suggests that in the same location, the abundance of viable initial inoculum varies from one season to another.

At the Mocuba trial on dwarf cashews the emi was 87.44% (year 2006) and 76.91% (year 2007), relatively lower than that of the common cashew trial (100.7% for 2006 and 86.94% for the year 2007) (Table 7.1). The eii on common cashew types was 1.4% in 2006 and 2.81 % in year 2007, both relatively higher than that on dwarfs, which were -0.41% and 1.45% for years 2006 and 2007 respectively.

At Pebane, common cashew trial, like Mocuba, the 2006 crop season was highly conducive to disease development (Fig. 7.2 G&H).

At Rapale, during the 2007 crop season the lowest value of emi (24.18%) was observed (Fig. 7.3), however the incidence–severity relationship remained robust as an exponential curve. However maximum incidence (12%) and severity (2.4%), of anthracnose was relatively lower compared to Mocuba, Nassuruma and Pebane trial sites. High emi values (disease spread) were consistently found in association with showers during the first week of July (Fig. 7:1; Fig.7:2 and Fig.7:3) regardless of location or crop season.

In general the emi of anthracnose on new cashew leaves was higher on the common cashew type than on dwarfs and this was supported by the eii which was also higher on common cashew trials.

Linear regression analysis of anthracnose incidence data from leaves and incidence data from nuts over three locations (Rapale, Nassuruma and Pebane) was not statistically significant (*P>0.001)* (data not presented).

7.5. General discussion

Both *C. gloeosporioides* and *C. acutatum* were targeted by PCR-technique but no evidence of presence of this last in Mozambique was detected. The hypothesis of multiple species of *Colletotrichum* being involved in cashew anthracnose as it happens in other crops such as almond, avocado, bell peppers and strawberry (Freeman *et al.*, 2000; Ivey *et al.*, 2004) was not supported at least with regard to *C. acutatum*. The slight variation in colony colour that was observed may be justified by the morphological and physiological heterogeneity known to occur within *C. gloeosporioides* (Lee *et al.*, 2005). For example, *C. gloeosporioides* isolates from mango, were found to exhibit different physiological responses to environmental conditions (Estrada *et al.*, 2000) and on Almond and Avocado, presence of pink and gray populations of *C. acutatum* has been the highlighted against consistently gray populations of *C. gloeosporioides* (Freeman *et al.*, 2000). A high degree of cultural variability among isolates of *C. gloeosporioides* from cashew was also reported from Brazil (Lopez and Lucas, 2010) in In the current study, instead of multiple species within the same genera causing the same disease, we found multiple species from different genera (*Fusarium*, *Pestalotia* and

Colletotrichum) associated in anthracnose disease symptoms. Therefore future recommendations on cashew anthracnose management in Mozambique, must address also these pathogens.

Numerous publications have dealt with the incidence-severity relationship of various pathosystems (Silva-Acuna, *et al*., 1999; Cardoso *et al*., 2004). Various models have been produced and their application and limitations were reviewed (McRoberts *et al*., 2003; Cardoso *et al*., 2004). In our study, the relationship between incidence and severity on cashew leaf anthracnose non-transformed data, best fitted the restricted exponential group model. This model curve was previously used by James & Shih (1973) on two different pathosystems (McRoberts *et al*., 2003). Limitations associated with practical use of incidence-severity relationships are essentially derived from their inconsistency in relations to location, season, epidemic stage, crop management systems and host genotype variations (Cardoso *et al*., 2004). Once the model has proven robust across all these, one may opt to use the easily measured parameter (incidence) (Sweetmore *et al*., 1994; Silva-Acuna *et al*., 1999; Cardoso *et al*., 2004). Therefore we recommend the use of leaf incidence in place of severity in genotype and fungicide screening trials, describing models for economic thresholds or epidemics studies of cashew leaf anthracnose. However, caution is needed since the cashew leaf anthracnose severity or incidence link to the panicle or nut anthracnose incidence/severity has not been established. We observed that the prevailing climatic (rainfall) conditions at flowering or fruiting stage play a major role in predicting leaf anthracnose before nut setting. This is in conformity with previous finding in Brazil where severity of anthracnose was coupled with rainfall and flushing of cashew (Cardoso *et al*., 2000).

At Rapale, were triadimenol fungicide was applied, both incidence and severity of anthracnose were reduced. This is confirms the efficacy of the product as previously referred (Freire *et al*., 2002).

In our model, we considered severity as independent variable and incidence as the dependent: Anthracnose is a polycyclic disease (Agrios, 2005). Changes in incidence over time are determined by the dynamics of severity or sources of inoculum at initial stages of epidemics (McRoberts *et al*., 2003). By exploring the regression curve minimum and maximum limits derived from the incidence-severity relationship, we have assessed the propensity of the environment for the disease epidemics across different sites, crop seasons, genotype

combinations and production system. Anthracnose spread was clearly associated with rainfall during the first week of July. In general this coincided with the flushing peak for most clones involved in the trials. This in agreement with knowledge that dispersion of anthracnose inoculum is by rain splashes (Ponte, 1984; Intini, 1987; Freire and Cardoso, 2003).

When the relationships between pairs of incidence and severity are mathematically expressed and consistent at multiple locations or environments, data from individual sites can be pooled into a summary equation without prejudice to proper interpretation (Cardoso *et al*., 2004). In this study overall means for essential coefficients such as eii and emi, were used to generate the summary equation that explained the relationships between anthracnose incidence and severity across different environments.

When incidence data of 1, 5, 10, 40 and 60% were computed in to the inverse equation**,** *S= (-1/a)*Ln (1-(1/b)*I)***,** severity estimates were returned as 0.01, 0.05, 0.10, 0.50 and 1% respectively. This indicates that very low levels of severity are associated with increased alloinfection.

In this model, both incidence and severity were found to increase. When incidence approaches a maximum of 80%, the severity is around 5%. Then, only severity continues to increase up to a maximum of 25%. This pattern of post-maximum incidence increase has been discussed by Cardoso *et al*. (2004). The spread of the disease may be limited because severely infected senescent leaves tend to drop off and the un-infected ones (20%) may reach maturity inhibiting fungal penetration.

In this study we adopted the scale developed by Nathaniels, (1996) initially used for cashew powdery mildew (*Oidium anacardii* Noack). In previous studies, cashew leaf anthracnose was assessed based on whole canopy scores (Anon., 1999; Cardoso *et al*., 1999; Cardoso *et al*., 2000; Cardoso *et al*., 2002), without standardized pictorial diagrams thus making it difficult to use by other workers. The use of diagrammatic scale decreased the absolute error of disease estimations by raters in the case of cashew leaf and nut blight patho-system (Menge[b] *et al*., 2013)

An adult common type cashew tree grows extensively towards the end of the rainy season and reproductively when the temperature declines (Wunnachit and Sedgley, 1992). Dwarfs and

young trees tend to grow continuously (Milheiro and Evaristo, 1994). Thus when the environment is favorable, two peaks of the disease epidemic may be observed in a year (Cardoso *et al*., 2000). Our method which is young leaf based has the advantage of being able to separate the two epidemics accurately. This method can be applied in all other tree crops with two flushes per year.

Estimation of cashew anthracnose damage through its incidence on young leaves has proven to be a more effective, faster, more accurate and user friendly method than severity scores. This is in line Sweetmore *et al*. (1994) who found incidence data to be simpler to collect and less subjective than severity and thus recommended for larger scale surveys. However, special attention may be necessary when assessing cashew anthracnose where other similar but distinguishable leaf diseases such as leaf blight (Sijaona *et al*., 2005) and Pestalotiopsis (Intini and Sijaona, 1983) are present. Furthermore, a recent review (Lewis and Ward, 2013), indicates that epidemiological data analysis could be improved through multivariate regression modeling.

References

Agrios, G.N. 2005. Plant Pathology. Fifth Edition. Elsevier Inc. 952 p.

Anon. 1999. Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew (*Anacardium occidentale* L.). Summary reports of European Commission supported STD-3 projects (1992-1995), published by CTA. [online http://intership.cta.int/pubs/std/vol2/pdf/221/pdf] 111-116, March 25, 2009.

Cardoso, J.E., Cavalcanti, J.J.V., Cavalcante, M. de J.B., Aragão, M. do L. & Filipe, E.M. 1999. Genetic resistance of Dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mold, and angular leaf spot. *Crop Protection 18:23-27*.

Cardoso, J.E., Felipe, E.M., Cavalcante, M. de J.B., Freire, F. das C.O. & Cavalcanti, J.J.V. 2000. Rainfall index and disease progress of anthracnose and black mold on cashew nut plants (*Anacardium occidentale*). *Summa Phytopathologica 26:413-16*.

Cardoso, J.E., Dos Santos, A.A., Freire, F.C.O., Viana, F.M.P., Vidal, J.C., Oliveira, J.N. & Uchoa, C.N. 2002. Monitorimento de doenças na cultura do cajueiro. Embrapa, Agroindustria Tropical, Fortealeza-CE.

Cardoso, J.E., Santos, A.A., Rossetti, A.G. & Vidal, J.C. 2004. Relationship between incidence and severity of cashew gummosis in semiarid north-eastern Brazil. *Plant Pathology 53:363-67*.

Carvalho, T. & Mendes, O. 1958. Doenças de Plantas em Moçambique. Departamento de Sanidade Vegetal, Instituto de Investigação Agronómica, Moçambique. 84 p

Chuang, T.Y. & Jeger, M.J. 1987. Relationship between incidence and severity of banana leaf spot in Taiwan. *Phytopathology 77:1537-41.*

Conticini, L. 1982. Guida fenologica dell'anacardio *(Anacardium occidentale* L.) *Rivista di Agricultura Subtropicale e Tropicale 86:221-42.*

Cornell, J.A. & Berger, R.D. 1987. Factors that influence the value of the coefficient of determination in simple linear and nonlinear regression models. *Phytopathology 77:63-70.*

Da Matta, E.A.F. & Lellis, W.T. 1973. Fungicidas e adubação no controle da "Queima do Cajueiro". *Boletim do Instituto Biologico de Bahia 12:37-40*.

Estrada, A.B., Dodd, J.C. & Jeffries, P. 2000. Effect of humidity and temperature o conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. *Plant Pathology 49: 608-618*.

Freire, F.C.O. & Cardoso, J.E. 2003. Doenças do cajueiro. Pages 192-225 *in*: Doenças de Fruteiras tropicais de interesse agroindustrial. Freire, F.C.O.; Cardoso, J.E. & Viana, F.M.P. (eds.). Embrapa Informação Tecnica. Brasilia.

Freire, F.C.O., Cardoso, J.E., Dos, Santos, A.A. & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21:489-94*.

Freeman, S., Minz, D., Jurkevitch, E., Maymon, M. & Shabi, E. 2000. Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopathology 90:608-614*.

Intini, M. 1987. Phytopathological aspects of cashew (*Anacardium occidentale* L.) in Tanzania. *International Journal of Tropical Plant Diseases 5:115-30*.

Intini, M. & Sijaona, M.E.R. 1983. Little known diseases of cashew (*Anacardium occidentale* L.) in Tanzania. *Revista di Agricoltura Subtropicale e Tropicale 77:421-29*.

Ivey, L., Nava-Diaz, M.L. & Miller, S.A. 2004. Identification and management of *Colletotrichum acutatum* on immature bell peppers. *Plant Disease 88*: *1198-1204*.

James, W.C. & Shih, C.S. 1973. Relationship between incidence and severity of powdery mildew and leaf rust on winter wheat. *Phytopathology 63:183-87*.

Lee, H.B., Park, J.Y. & Jung, H.S. 2005. Identification, growth and pathogenicity of *Colletotrichum boninense* causing leaf anthracnose on Japanese Spindle Tree. *Plant Pathology journal 21: 27-32*.

Lewis, F. & Ward, M. 2013. Improving epidemiological data analysis through multivariate regression modeling. *Lewis and Ward Emerging Themes in Epidemiology 10:4. http://www.ete-online.com/content/10/1/4. December, 2013*.

Lopez, A.M.Q. and Lucas, J.L. 2010. Colletotrichum isolates related to anthracnose of cashew trees in Brazil: Morphological and Molecular description using LSU and rDNA sequences. *Braz. Arch. Biol.Tecnol. 53:741-752*.

Menge[a], D., Makobe, M.N., Shomari, S. & Tiedemann, V. 2013. Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. Causing leaf and nut blight (*Anacardium occidentale* L.). *International Journal of Advanced Research 1:26-38*.

Menge[b], D., Makobe, M.N., Shomari, S. & Tiedemann, V. 2013. Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. Causing leaf and nut blight (*Anacardium occidentale* L.). *Journal of Yeast and Fungal Research 4:12-20*.

Masawe, P.A.L.; Cundal, E.P. & Caligari, P.D.S. 1997. Powdery mildew (*Oidium anacardii*) onset and development on flowering panicles of cashew clones (*Anacardium occidentale* L.) as a measure of clone resistance. *Tropical Agriculture 79:229-34.*

McRoberts, N., Hughes, G. & Madden, L.V. 2003. The theoretical basis and practical application of relationships between different disease intensity measurements in plants. *Annals of applied Biology 142:191-11*.

Menge[a], D., Makobe, M.N., Shomari, S. & Tiedemann, V. 2013. Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. Causing leaf and nut blight (*Anacardium occidentale* L.). *International Journal of Advanced Research 1:26-38*.

Menge[b], D., Makobe, M.N., Shomari, S. & Tiedemann, V. 2013. Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. Causing leaf and nut blight (*Anacardium occidentale* L.). *Journal of Yeast and Fungal Research 4:12-20*.

Milheiro, A.V. & Evaristo, F.N. 1994. Manual do cajueiro. Cultivar. Associação de Tecnicos de Culturas Tropicais. Porto. 204 p.

Muniz, M.F.S., Lemos, E.E.P., Varzea, V.M.P., Rodrigues, C.J.Jr. & Bessa, A.M.S. 1998. Characterization of *Colletotrichum gloeosporioides* (Penz.) Sacc. Isolates and resistance of cashew (*Anacardium occidentale* L.) to the pathogen. Pages: 249-253, *in*: Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds.). Biohybrids International Ltd., Reading.

Nathaniels, N.Q.R. 1996. Short communication. Methods, including visual keys for assessment of cashew powdery mildew (*Oidium anacardii* Noack) severity. *International Journal of Pest Management 42:199-05*.

Ohler, J.G. 1979. Cashew. Koninklijk Instituut voor de Tropen. Amsterdam. 260 p.

Ponte, J.J. 1984. Doenças do cajueiro no Nordeste Brasileiro. Documento 10. EMBRAPA-Departamento de Difusão de Tecnologia. Brasilia. 51 p.

Rouse, D.I., Mackenzie, D.R.; Nelson, R.R. & Elliott, V.J. 1981. Distribution of wheat powdery mildew incidence in field plots and relationship to disease severity. *Phytopathology 71:947-50*.

Sijaona, M.E.R. & Mansfield, J.W. 2001. Variation in the response of cashew genotypes to the targeted application of fungicide to flower panicles for control of powdery mildew disease. *Plant Pathology 50:224-248.*

Sijaona, M.E.R., Reeder, R.H. & Waller, J.M. 2005. Cashew leaf and nut blight-A new disease of cashew in Tanzania caused by Cryptosporiopsis spp. *Plant Pathology 55:576-576.*

Silva-Acuna, R., Maffia, L.A., Zambolim, L. & Berger, R.D. 1999. Incidence-Severity Relationships in the Pathosystem Coffee arabica-*Hemileia vastatrix. Plant Disease 83:186-188.*

Snedecor, G.W. & Cochran, W.G. 1980. Statistical methods (7th Edition). Ames: Iowa State University Press. 807 p.

Sweetmore, A., Simons, S.A. & Kenward, M. 1994. Comparison of disease progress curves for yam anthracnose **(***Colletotrichum gloeosporioides***)**. *Plant Pathology 43:206-215*.

Whitelaw-Weckert, M.A., Curtin, S.J., Huang, R., Steel, C.C.; Blanchard, C.L. & Roffey, P.E. 2007. Phylogenetic relationships and pathogenicity of Colletotrichum acutatum isolates from grape in subtropical Australia. *Plant Pathology 56:448-463*.

Wunnachit, W. & Sedgley, M. 1992. Floral structure and phenology of cashew in relation to yield. *Journal of Horticultural Science 67:769-77*.

Table 7.1: General characteristics of the trial sites in which cashew anthracnose incidence and severity data were collected in Mozambique during 2006 and 2007.

Trial site	Distance from Nassuruma(km)	Type of grafted cashew progenies	Number of Cultivars	Plant spacing(m)	Plant Age (years)	Owned by
Nassuruma		dwarf progenies	10	8x6	9	IIAM *
Mocuba	460	dwarf progenies	39	10x10	7	NGO **
Mocuba	460	common progenies	33	10x10	7	NGO
Pebane	512	dwarf progenies	67	10x10	8	INCAJU***
Pebane	512	common progenies	80	10x10	8	INCAJU

* Agriculture Research Institute of Mozambique; ** Non-governmental organization;

*** National Institute for Cashew Development

Table 7.2: Regression equations of Incidence (I) on Severity (S) of leaf anthracnose (*Colletotrichum gloeosporioides* Penz.) under different environments and different cashew *(Anacardium occidentale* L.) genotypes in Mozambique, 2006 -2007.

Restricted exponential function		I =	b*	(1- e**(-a*S))			Pecentage variance	
Location	**Type germplasm**	**Year**	**b**	**K**	**a=(K + b)**	**R**	**Accounted for**	**SE**
Nassuruma	dwarf progenies	2006	68.56	(-59.82)	8.74	0.8595	82.5	6,340
Nassuruma	dwarf progenies	2007	123.00	(-120.00)	3.00	0.9404	92.5	3,420
Rapale	dwarf & common	2007	24.2	(-23.6)	0.60	0.726	91.3	1,090
Mocuba	dwarf progenies	2006	87.44	(-87.85)	(-0.41)	0.8650	74.5	9,860
Mocuba	dwarf progenies	2007	76.91	(-75.46)	1.45	0.8056	84.8	6,950
Mocuba	common progenies	2006	100.7	(-99.3)	1.40	0.8775	82.5	7,710
Mocuba	common progenies	2007	86.94	(-84.13)	2.81	0.8569	86.8	7,373
Pebane	dwarf progenies	2006	78.71	(-79.06)	(-0.35)	0.765	68.2	14,200
Pebane	dwarf progenies	2007	55.40	(-56.32)	(-0.92)	0.6608	79.8	9,380
Pebane	common progenies	2006	88.26	(-87.83)	0.43	0.7980	84.6	13,100
Pebane	common progenies	2007	85.22	(-86.24)	(-1.02)	0.7402	88.1	12.1
Overall mean			**79.58**	**(-78.15)**	**1.43**	**0.8086**	**83.2**	**8,320**

Regression equation of incidence applied for each location: I = b* (1- e**(-a*S)); SE= Standard error of observations; R= Coefficient of determination; b= Estimated maximum incidence; a=Estimated initial incidence; * multiply; ** power to; For all locations P***.<0.001.

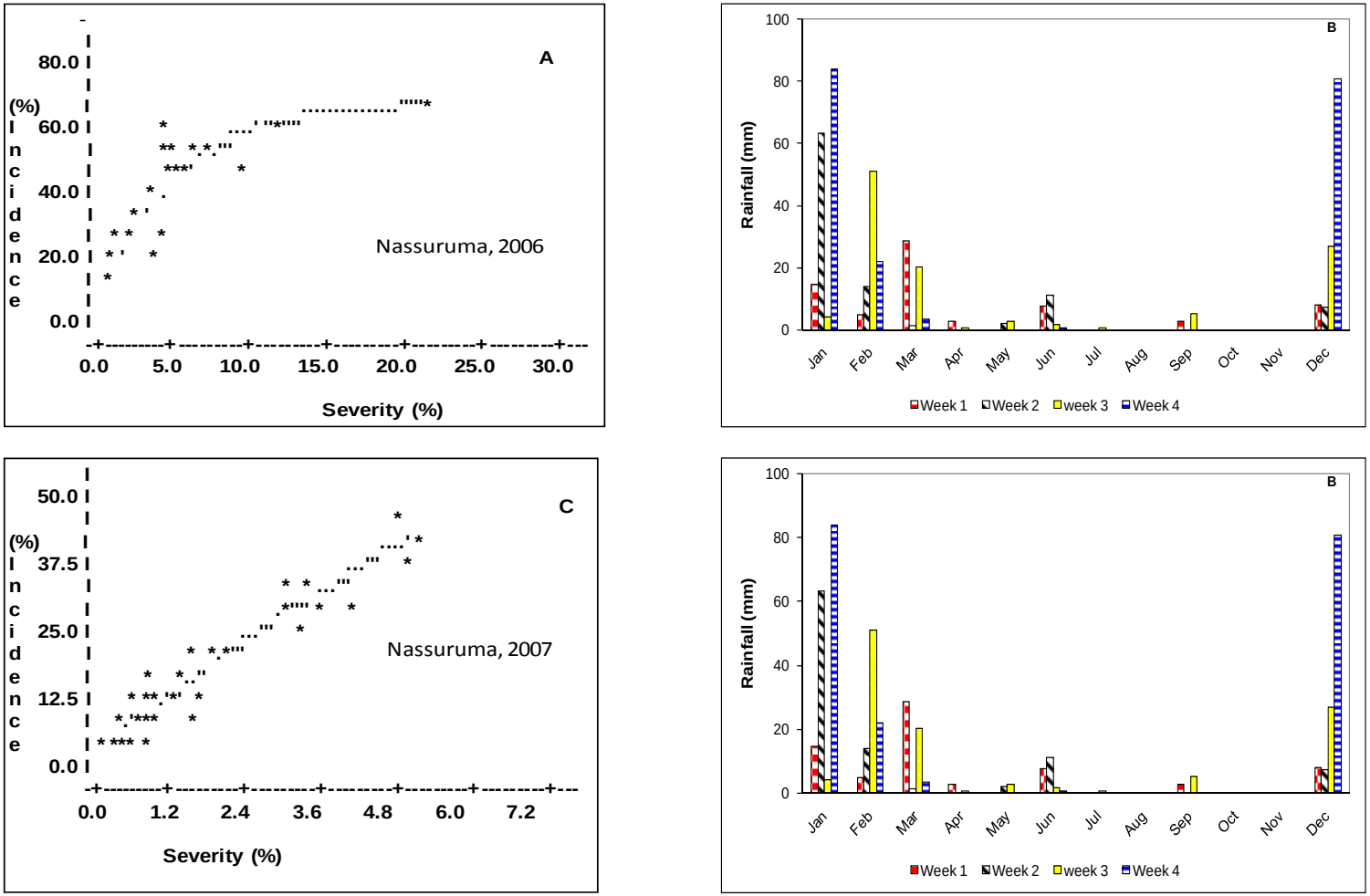


Figure 7.1: Cashew anthracnose severity and incidence relationships on dwarf genotypes and rainfall distribution over two years at Nassuruma, Mozambique.

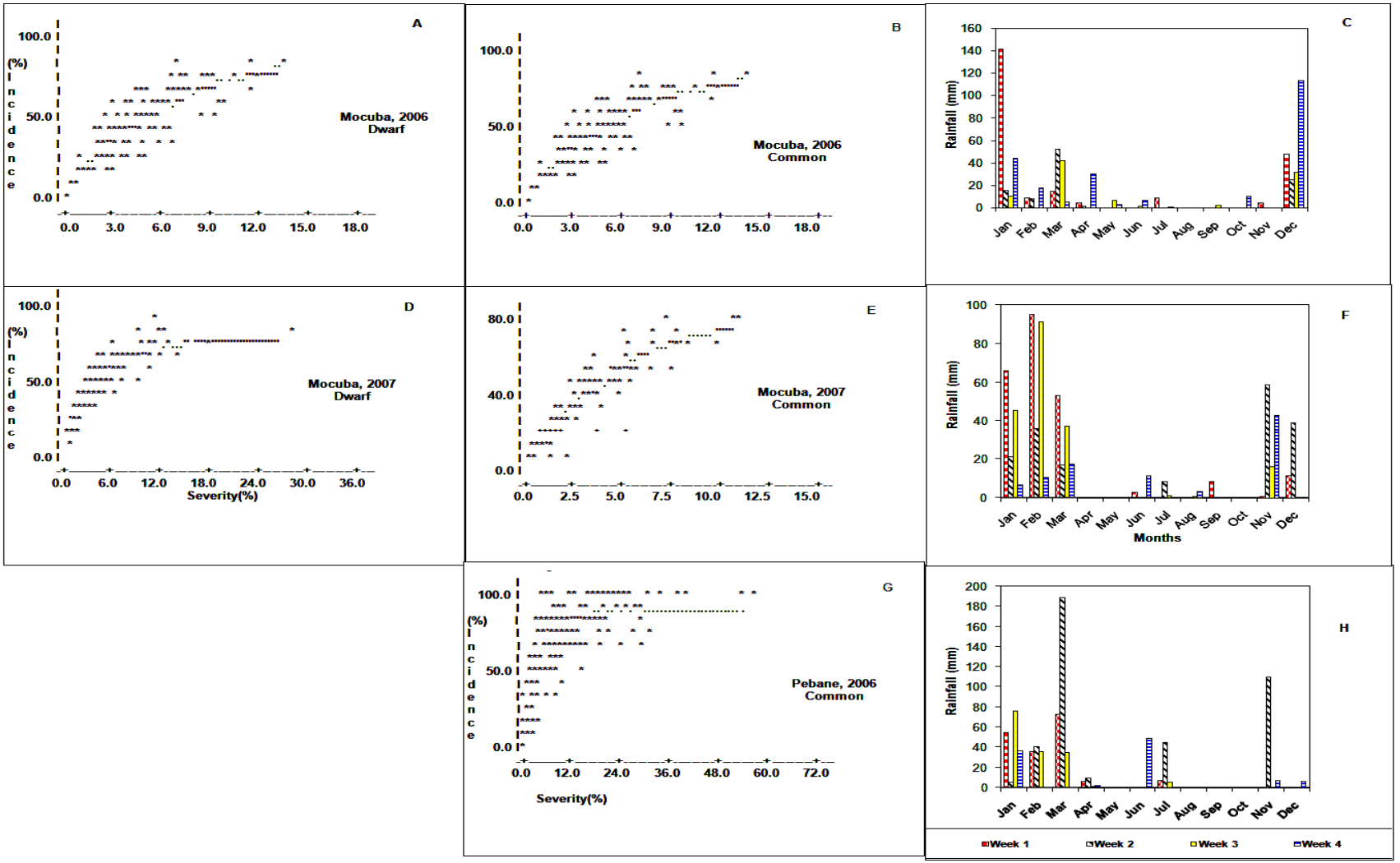
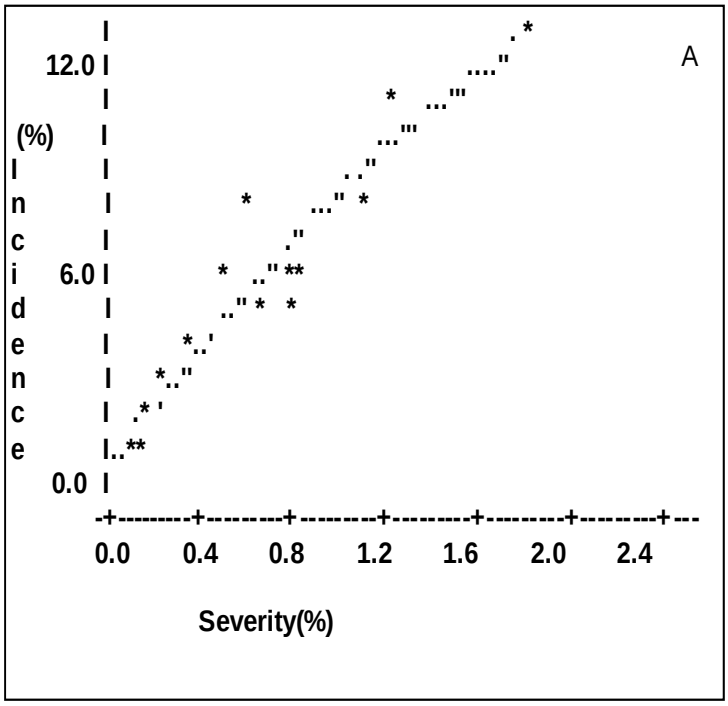


Figure 7.2: Cashew anthracnose severity and incidence relationships on multiple genotypes, years and locations in Mozambique.



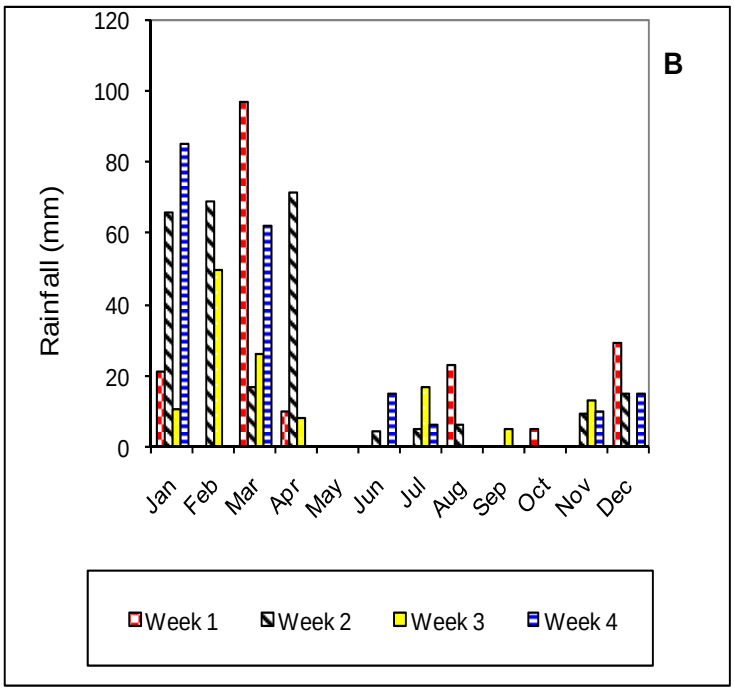


Figure 7.3: Cashew anthracnose severity and incidence relationships (A) on and rainfall distribution (B) at Raple, Mozambique.

CHAPTER VIII: A SEARCH FOR ANTHRACNOSE RESISTANT CASHEW CULTIVARS IN MOZAMBIQUE

8.1. Abstract

Host resistance is a key aspect among disease control strategies. Dwarf and giant cashew genotypes were separately screened for resistance against anthracnose. Disease incidence was assessed on emerging leaves over three consecutive crop season in tree sites of northern Mozambique. Leaf incidence is presented as new field method in screening cashew genotypes for resistance to anthracnose. It is fast, precise and consistent in ranking cultivars after three seasons. Seasonal, cultivar, incidence means were comparable using Fishers' LSD test. The method enabled us to differentiate highly infected cultivars from those consistently less infected across seasons and locations. No single vertically resistant clone was identified out of 229 entries. However, hierarchical tables of clonal sensitivity ranked cultivars 1.12PA, 12.8PA and 1.18PA as tolerant and 11.9PA and 2.3BG as susceptible among the dwarfs. Among the giant genotypes, cultivars NA7, MB77, 1.5R and MCH-2 ranked tolerant and IM1 and MU3 susceptible. Tolerant clones were therefore recommended to integrate into breeding programmes as strategic cultivars for development of durable resistance to cashew anthracnose. Similarly, clones such as 2.5VM, 1EM, MB75 and others that revealed incidence consistency over seasons can be used as standards in screening trials regardless of their level of susceptibility/tolerance[3].

[3] **Article published in *Crop Protection 50 (2013): 6-11***

8.2. Introduction

Cashew, *Anacardium occidentale* L. is a crop with demonstrated potential for foreign exchange and job creation throughout the world (Cardoso *et al*., 1999; Freire *et al*., 2002). In Mozambique alone, cashew supports more than one million small farmers, over six thousand employees and it channels over 20 million US dollars per year (Anon. 2007).

It was during the XVIth century, that European travelers introduced the cashew from Brazil in a form of seed (Milheiro and Evaristo, 1994; Behrens, 1996). In Mozambique, later introductions are recorded from the 70's and 90's, yet as seeds progenies of Brazilian CCP09, CCP76, CCP1001 and Matriz 96 genotypes. Seed introductions were also made from India and Zambia (Prasad *et al*., 2000) and most recently, from Tanzania. Continuous planting of cashew via seed returned heterozygous orchards throughout Asia, Africa and Latin America (De Araujo and Da Silva, 1995) with heterogeneous sensitivity to diseases.

Anthracnose disease has become seriously damaging in Mozambique (Dhindsa and Monjana, 1984). Tolerant genotypes have been identified in Brazil (Cardoso *et al*., 1999), Guiney Bissau and Cameroon (Anon. 1999) and Tanzania (Intini, 1987). But, importation of tolerant clones is limited by international regulations on trans-movement of germplasm. In addition, variation on pathotypes and environmental conditions cannot ensure safety to the imported material. Therefore the objective of this article was to identify, among locally available germplasm, clones that are resistant to anthracnose.

8.3. Material and methods

8.3.1. Locations and experimental design

The orchard for this work consisted of a range of cloned dwarf and common cashew types. The trial sites are indicated in Fig.8.1. At Mocuba and Pebane sites, the trials, common and dwarf, were established parallel to one another with only six meters road between the two. At Nassuruma cashew research station, in Meconta district, only one trial consisting of dwarf progenies was considered for this study.

All the trials were laid out in randomized complete block design (Gomez and Gomez, 1984) with cultivar as the treatment. Each trial consisted of three replications of three plants each. Other details on the orchards are provided in Table 8.1.

8.3.1.1. Field data collection and statistical treatment

For each growing season, five shoots from each, the north and south sides of individual trees, were tagged with a sisal cord. This intended to visualize the shoots and facilitate repeated scoring (Masawe *et al*., 1997). Disease assessments were made for three consecutive crop seasons from 2006 beginning in May or June and ending in September as per the development and maturation of new flushes. Weekly or two weekly intervals were considered depending on size of the trial. From individual shoots, all emerging leaves from the very same crop season were counted and each assessed for presence or absence of anthracnose necrotic lesions. Readings were made up to the tenth leaf whenever shoots grew beyond. Therefore in this trial incidence reflected the proportion of visually diseased leaves (percentage) (McRoberts *et al*., 2003). Leaf severity scores were recorded from the Nassuruma trial based on the scale developed by Nathaniels (1996) for powdery mildew which has been further detailed by Sijaona *et al*., (2001) and was found practical also for anthracnose necrosis evaluation.

Disease scores were initially processed to return plant mean scores as detailed by Masawe *et al*., (1997). For individual cropping season incidence data were tabulated in excel spreadsheet per location, date of observation, replicate, cultivar and plant. Data were analyzed using the statistical program GenStat (2003). Analysis of variance (ANOVA) was used to test differences between the disease incidence responses of cultivars per cropping season. The data were acceptably normal with heterogeneous treatment variances. Thus, Fishers'protected t-test of least significant difference (LSD) at 1 or 5 % levels of significance was used to separate incidence means (Snedecor and Cochram, 1980) for each year. At Nampula trial, data were log transformed before mean separation. Annual means were ranked by giving numbers from the smallest to the largest. An overall mean was calculated as the sum of cultivar ranks divided by the number of seasons (3). Final ranking of cultivars was made on cultivar overall mean.

8.4. Results

8.4.1.1. Cloned dwarf progenies

At Nassuruma, cashew genotypes' reaction to anthracnose infection was variable over the years and clones (Table 8.2a). Overall ranks indicate that clone 11.8PA expressed lowest levels of anthracnose leaf incidence varying from 5 to 21 % over the years while clone 11.9PA ranked high with incidence levels varying from 35 to 66% (Table 8.2a).

Incidence and severity relationships on leaf anthracnose over crop seasons, germplasm variation, locations and fungicide spray production systems have been established (subject for specific publication). Field data proved to be robust for the use of disease incidence as a valuable parameter for screening germplasm instead of severity which is difficult to assess. Table 8.2b illustrates the similarity between outputs for clonal ranking and disease severity and anthracnose incidence data.

At Mocuba, overall ranking of cloned cashew dwarf genotypes indicated that clone 12.8PA expressed lowest levels of anthracnose leaf incidence varying from 23 to 34 % over the years while clone 2.3BG ranked high with incidence levels varying from 49 to 79% (Table 8.3).

On dwarf and cloned genotypes at Pebane, anthracnose disease incidence was also variable from year to year within each clone. However, overall ranking indicated that clone 29EM had the lowest incidence rank, contrasting with clone 9EM on which anthracnose incidence ranked highest (Table 8.4).

8.4.2. Cloned common progenies

On common and cloned genotypes at Mocuba, anthracnose disease incidence was also variable from year to year within each clone. Overall ranking positioned two of the clones (clone NA7 and MB77) with least leaf anthracnose incidence. Clone IM1, in contrast ranked highest with incidence between 65 and 84% over the years (Table 8.5).

At Pebane, overall ranking of cloned cashew common genotypes indicated that clone 1.5R and clone MCH-2 expressed lowest levels of anthracnose leaf incidence varying from 2 to 16 % and 0 to 37 respectively (Table 8.4). Highest scores of anthracnose incidence were recorded from clone MU3, varying from 53 to 68% (Table 8.6).

At Pebane and Nassuruma, among the dwarf progenies, clones 2.5VM, 1EM, 1.12PA and 11.8PA were the most consistent tolerant clones. In contrast, clones 5.9PA, 17PA, 12PA, 7.10PA, and 11.9PA were consistently susceptible over the experimental period. All other clones varied in incidence levels highly from one season to another (Table 8.3). Among the common progenies, consistency on anthracnose incidence was observed only in susceptible clones, NA1001, MU1, MU3, MB75 and IM1 at Mocuba trial (Table 8.5).

Some clones were integrated at two trial sites. Anthracnose incidence overall ranks have located most of them consistently either as tolerant on top or susceptible at bottom of the positioning table: Clones 1.12PA and 11.8PA at Nassuruma trial ranked first and second from the top (Table 8.2a). Similarly, at Pebane trial, these clones ranked third and fourth from top (Table 8.4). At Mocuba the same clones were intermediate (Table 8.6). Clones 5.12PA and 11.7PA were intermediate at Nassuruma (Table 8.5) and Mocuba (Table 8.6) and towards the bottom at Pebane (Table 8.4). The rank changes for a particular cultivar can be explained by means of the LSD value as compared to the most susceptible or the most tolerant clones in the trial. Therefore, in this method, a give clone could be categorized as “tolerant”, if not statistically different from the top reference, “susceptible” if not different the bottom reference and “intermediate” if different from either of the above references.

8.5. Discussion

For small scale farmers, resistant cultivars are still the best option for integrated crop management (Cardoso *et al*., 1999). Unlike other methods, they provide suitable and environmentally safe control of the disease. In addition, resistance to *Colletotrichum* diseases has often been shown to be of polygenic and durable nature (Waller, 1992). In this study 229 cultivars among dwarfs and common genotypes have been hierarchically ranked as per their susceptibility to anthracnose disease. Less susceptible clones can be considered for analytic selection in conjunction with yield and powdery disease information.

We found variability of cashew cultivars to anthracnose disease development. This is in conformity with previous findings where variability on cashew reaction to anthracnose pathogen was demonstrated both *in vitro* (Muniz *et al*., 1997; Anon. 1999) and in field (Cardoso *et al*., 1999). The disease is known to vary in severity and aggressiveness with the prevailing environmental conditions (Cardoso *et al*., 2000; Topper *et al*., 2003). The results of the current investigations are the first to be obtained in Mozambique.

Cashew germplasm screening generally uses severity data and this has been previously achieved in field (Cardoso *et al*., 1999) and *in vitro* (Muniz *et al*., 1998; Anon. 1999) studies. This is because the relationship between incidence and severity had not been established at the time that these previous studies were being carried out. In fact the idea of having it established in most crops is to reduce labour cost and improve accuracy (McRoberts *et al*., 2003). More importantly, the whole plant scores currently used in cashew anthracnose assessment (Cardoso *et al*., 2000) can lead to misleading conclusions because under severe attack, highly susceptible clones become defoliated (Freire *et al*., 2002) and therefore there is an increasing risk such a defoliated clone could be underscored. Tolerant clones, however, can be scored taking into account multiple seasons' damage caused by the pathogen. Using the incidence approach, anthracnose incidence values can be used to assess two epidemics within a single calendar year: cashew vegetative growth epidemic and cashew generative growth epidemic and thus correlate them for possible disease forecast model.

In Mozambique, there has been a general perception that dwarf genotypes are highly susceptible to anthracnose as compared to common ones. At Mocuba as well as Pebane, dwarf and common genotypes were established in parallel to one another and incidence data do not support such an observation. The height and diameter of common types could well be obscuring the damage caused by anthracnose to casual observers.

Most of the germplasm used in the present study are among the genetically elite material for the northern region. They represent recent introductions from Brazil (Entreposto Monapo-collection); selection from Nampula Agronomic Research Station (Posto Agronomico-Collection); selection from Nassuruma cashew research station (Nassuruma-collection) and farmers' field collections referred to by the names of location sources and numbers, e.g. NC4 is the fourth elite mother tree from Naburi-Calima. From all these, no evidence of source

related tolerance/resistance was noted. This supports the concept of genetically controlled cashew reaction to infection (Waller, 1992).

Reaction of dwarf cashew clones to *C. gloeosporioides* isolates has been studed in controlled environment by Lopez and Lucas (2010) and variation in both host susceptibility and pathogen aggressiveness were found. Host structural barriers such as exocarp cuticle thickness and reduced number of stomatal pores were associated with resistance.

Dwarf clones 1.12PA and 11.8PA and common clones, NA7, MB77, 1.5R and MCH-2, were grouped as tolerant to anthracnose disease. Likely, heritability of resistance on cashew disease has been highlighted from recent studies carried out in Tanzania (Sijaona *et al*., 2001) therefore these clones are potentially relevant for cashew breeding programmes in Mozambique **(**Freire *et al*., 2002). However, Clone 11.9PA (dwarf progeny), MU3 and IM1 (Common progenies) were isolated as the most susceptible and therefore they can be integrated as positive control in trials elsewhere. Regardless of their level of susceptibility/tolerance, clones such as 2.5VM, 1EM, NA1001, MU1, MU3 and others have shown seasonal consistency can be recommended as standard in screening trials.

References

Anon. 1999. Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew (*Anacardium occidentale* L.). Summary reports of European Commission supported STD-3 projects (1992-1995), published by CTA. [online http://intership.cta.int/pubs/std/vol2/pdf/221/pdf] 111-116, March 25, 2009.

Anon. 2007. Reunião Anual da African Cashew Alliance (ACA). Subsector do Caju em Moçambique: Evolução e Perspectivas. htt/www/africancashewalliance.com . March 23, 2008.

Behrens, R. 1996. Cashew as an Agro-forestry Crop. Prospects and Potentials. Tropical Agriculture 9. Gmbh: Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) Gmbh, Eschborn. 83 p.

Cardoso, J.E., Cavalcanti, J.J.V., Cavalcante, M de J.B. Aragão, M. do L. & Filipe, E.M. 1999. Genetic resistance of dwarf cashew (*Anacardium occidentale* L.) to anthracnose, black mold and angular leaf spot. *Crop Protection 18: 23-27.*

Cardoso, J.E., Felipe, E.M., Cavalcante, M. de J.B., Freire, F. C.O. & Cavalcanti J.J.V. 2000. Rainfall index and disease progress of anthracnose and black mold on cashew nut plants (*Anacardium occidentale*). *Summa Phytopathologica 26: 413-16*.

De Araujo, J. & Da Silva, V.V. 1995. Cajucultura. Modernas Tecnicas de Produção. Ministerio da Agricultura, do Abastecimento e da Reforma Agraria. Empresa Brasileira de Pesquisa Agropecuaria - EMBRAPA. Centro Nacional de Agroindustria tropical-CNTP, Fortaleza, Brazil. 292 p.

Dhindsa, P.P. & Monjana, A.M. 1984. Index of plant diseases and associated organisms of Mozambique. *Tropical Pest Management 30: 407-429.*

Freire, F.C.O., Cardoso, J.E., Dos Santos , A.A & Viana, F.M.P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. *Crop Protection 21: 489-494*.

Gomez, A.K. & Gomez, A.A., 1984. Statistical procedures for agricultural research. Second edition. John Wiley & Sons, Inc., New York. 680 p.

Intini, M. 1987. Phytopathological aspects of Cashew (*Anacardium occidentale* L.) in Tanzania. *International Journal of Tropical Plant Disease 5: 115-130*.

Lopez, A.M.Q. & Lucas, J.A., 2010. Reaction of dwarf cashew clones to Colletotrichum gloeosporioides isolates in controlled environment. *Sci Agric. 62:228-235.*

Masawe, P.A.L., Cundal, E.P. & Caligari, P.D.S. 1997. Powdery mildew (*Oidium anacardii*) onset and development on flowering panicles of cashew clones (*Anacardium occidentale* L.) as a measure of clone resistance. *Tropical Agriculture (Trinidad.) 79: 229-324.*

McRoberts, N., Hughes, G. & Madden, L.V. 2003. The theoretical basis and practical application of relationships between different disease intensity measurements in plants. *Annals of Applied Biology 142: 191-211*.

Milheiro, A.V. & Evaristo, F.N., 1994. Manual do Cajueiro (Cashew Hand Book). Cultivar. Associação de Técnicos de Culturas Tropicais, Porto, Portugal. 204 p.

Muniz, M.F.S., Lemos, E.E.P., Varzea, V.M.P., Rodrigues, C.J.Jr. & Bessa, A.M.S. 1998. Characterization of *Colletotrichum gloeosporioides* (Penz.) Sacc. Isolates and resistance of cashew (*Anacardium occidentale* L.) to the pathogen. Pages: 249-253, *in*: Proceedings of the International cashew and Coconut conference 17-21 February, 1997. Topper, C.P., Caligari, P.D.S., Kullaya, A.K., Shomari, S.H., Kasuga, L.J., Masawe, P.A.L. & Mpunami, A.A. (eds.). Biohybrids International Ltd., Reading.

Nathaniels, N.Q.R. 1996. Short communication. Methods, including visual keys for assessment of cashew powdery mildew (*Oidium anacardii* Noack) severity. *International Journal of Pest Management 42: 199-205*.

Prasad, M.V.R., Langa, A. & Consolo, J.P. 2000. Selection of elite cashew genetic material in Mozambique. *The cashew 14: 8-23.*

Sijaona, M.E.R., Clewer, A., Maddison, A. & Mansfield, J.W. 2001. Comparative analysis of powdery mildew development on leaves, seedlings and flower panicles of different genotypes of cashew. *Plant Pathology 50: 234-243.*

Snedecor, G.W. & Cochran, W.G. 1980. Statistical methods (7th edition). Iowa State University Press, Ames, Iowa. 807 p.

Topper, C.P., Boma, F., Sijaona, M.E.R. & Anthony, J.K. 2003. Crop Protection in cashew farming systems. Pages: 138 -172, *in*: Knowledge transfer for sustainable tree crop development. A case history of the Tanzanian Integrated Cashew Management Programme. Topper, C.P. & Kasuga, L.J. (eds). BioHybrids Agrisystems Ltd., Reading.

Waller, J.M. 1992. *Colletotrichum* diseases of perennial and other cash crops. Pages 166-185, *in: Colletotrichum: Biology, pathology and control*. Bailey, J.A . & Jeger, M.J. (eds). CABI, British Society for Plant Pathology, Wallingford.

Table 8.1: General characteristics of the trial sites in which cashew anthracnose incidence and severity data were collected in Mozambique during 2006 and 2007

Year	2006		2007		2008		
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Overall Rank
NA7	26.56	8	6.06	1	22.52	1	3,3
MB77	27.57	10	14.78	3	24.49	3	3,3
MU2	24.86	6	18.96	5	45.74	12	5,3
103,82	20.83	4	34.68	14	45.60	11	7,7
103,81	9.80	1	19.72	7	65.83	23	9,7
Na100	40.43	21	17.28	4	38.52	6	10,3
MB76	40.40	20	27.12	9	30.15	4	10,3
1.5R	31.00	15	49.94	22	23.77	2	11,0
MU63	29.87	13	40.91	17	44.22	9	13,0
MS-2	23.13	5	42.39	18	57.13	18	13,0
02NASS	29.80	12	13.34	2	75.29	28	13,7
EBA70	37.77	17	21.60	8	55.71	17	14,0
MU106	41.67	22	28.78	12	42.94	8	14,0
NA96	33.03	16	34.61	13	45.94	13	14,0
NA98	29.43	11	35.40	15	52.93	16	14,0
PPE13	34.13	18	28.63	11	50.00	15	14,0
103,79	55.07	30	27.86	10	39.74	7	14,7
MU45	24.87	7	43.57	19	63.52	22	15,7
IM5	52.30	28	38.21	16	32.79	5	16,0
103,68	45.37	24	51.13	24	45.03	10	16,3
MU18	17.40	2	58.02	29	73.10	27	19,3
MU42	37.63	19	54.17	25	46.05	14	19,3
103,85	27.07	9	60.51	30	59.37	21	19,3
NA5	18.00	3	54.69	26	76.65	31	20,0
MU32	49.06	27	19.44	6	75.36	29	20,0
NC3	30.97	14	50.61	23	68.92	25	20,7
NC1	48.67	26	47.32	20	58.71	20	20,7
MB83	47.53	25	56.48	27	57.46	19	22,0
NA1001	55.40	31	48.20	21	68.52	24	23,7
MU1	42.40	23	69.28	33	75.50	30	25,3
MU3	72.33	33	57.55	28	69.74	26	28,7
MB75	54.76	29	65.01	32	78.82	32	29,0
IM1	65.06	32	61.35	31	84.58	33	31,0
SEM	7.63		8.34		3.75		
LSD	28.72		23.61		14.07		
CV(%)	35.7		36.7		11.9		
F.level (%)	1.0		5.0		1.0		

* Agriculture Research Institute of Mozambique; ** Non-governmental organization; *** National Institute for Cashew Development

Table 8.2a: Comparison of cashew anthracnose leaf incidence (%) on dwarf cashew progenies at Nassuruma, Mozambique, 2006-2008

Year	2006		2007		2008		
Identifier	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Overall Rank**
11.8PA	21.59(58.9)	2	4.77(3.73)	1	8.30(4.09)	2	1.7
1.12PA	20.92(29.2)	1	11.79(8.21)	4	3.83(5.68)	1	2.0
1.3PA	34.38(87.1)	4	8.74(16.16)	2	9.66(111.08)	3	3.0
2.3A	34.26(301.4	3	9.43(10.55)	3	12.17(26.70)	4	3.3
5.12PA	35.46(71.8)	5	16.71(29.74)	6	13.92(60.96)	6	5.7
2.4PA	42.98 (9.9)	7	25.23(51.21)	8	13.84(53.98)	5	6.7
2.5VM	43.28(379.7)	8	15.37(82.30)	5	22.75(216.22)	8	7.0
11.7PA	47.00(252.6)	9	21.36(106.16)	7	18.82(97.74)	7	7.7
7.10PA	41.38(291.3)	6	39.48(70.14)	10	33.99(127.42)	9	8.3
11.9PA	50.07(441.5)	10	35.24(53.56)	9	66.40(119.35)	10	9.7
SEM	8.59		3.82		5.22		
LSD	NS		15.66		21.39		
C V (%)	39.9		35.2		44.4		
Fishers' level (%)			1		1		

SEM = Standard error mean, LSD = Least significant difference, C V (%) = Percentage coefficient of variance, ( ) = Log transformed percentages

Table 8.2b: Comparison of cashew anthracnose leaf incidence (%) on dwarf cashew progenies at Nassuruma, Mozambique, 2006-2008

Year	2006		2007		2008		
Identifier	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Overall Rank**
1.12PA	1,403	1	1,237	3	0.817	1	1.7
11.8PA	1,447	2	0.313	1	0.947	2	1.7
1.3PA	4,347	6	0.907	2	1,017	3	3.7
2.3PA	3,480	3	1,450	4	1,813	5	4.0
5.12PA	4,117	4	1,583	5	1,890	6	5.0
11.7PA	4,143	5	2,367	7	4,043	7	6.3
2.4PA	5,420	8	3,580	8	1,165	4	6.7
2.5VM	4,717	7	1,890	6	4,643	8	7.0
7.10PA	8,240	9	5,703	10	4,910	9	9.3
11.9PA	12,807	10	4,833	9	18,410	10	9.7
SEM	2,659		0.687		2,422		
LSD	NS		2,814		9,928		
C V (%)	83		50.1		106.1		
Fishers' level (%)			1		1		

SEM = Standard error mean, LSD = Least significant difference, C V (%) = Percentage coefficient of variance, ( ) = log transformed percentages

Table 8.3: Comparison of cashew anthracnose leaf incidence (%) on dwarf cashew progenies at Mocuba, Mozambique, 2006-2008

Year	2006		2007		2008		Overall
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Rank
12.8PA	32.87	8	33.93	5	22.45	2	5.0
6.7NASS	22.46	2	40.71	12	44.04	10	8.0
4.1AD	24.87	3	34.80	6	60.66	21	10.0
11.7PA	24.90	4	55.42	24	23.46	3	10.3
1.20VM	22.41	1	55.26	23	37.80	7	10.3
3.2VM	28.73	6	30.22	2	64.57	24	10.7
12.1PA	42.90	18	38.75	11	27.45	4	11.0
35EM	43.90	19	36.04	9	51.73	16	14.7
2.7NASS	33.90	11	54.59	21	45.01	12	14.7
7EM	33.13	9	22.11	1	77.99	35	15.0
12.3PA	26.43	5	59.47	32	38.21	8	15.0
11.8PA	44.23	20	54.94	22	30.27	5	15.7
2.3PA	46.60	22	35.33	8	56.79	18	16.0
5.12PA	42.47	17	31.68	4	72.91	32	17.7
2EM	52.30	27	49.93	20	32.12	6	17.7
12.9PA	39.31	15	34.80	7	71.69	31	17.7
2.5VM	35.47	13	48.15	19	62.45	22	18.0
10.1NASS	48.07	24	59.96	33	10.03	1	19.3
12.2PA	33.37	10	71.91	38	44.49	11	19.7
11.2PA	32.63	7	64.82	36	55.80	17	20.0
1.20VM	40.07	16	55.90	25	59.08	19	20.0
1.18VM	55.61	29	30.33	3	69.85	30	20.7
12.6PA	53.70	28	56.11	26	40.74	9	21.0
16.1BG	64.04	36	47.11	18	50.09	15	23.0
5EM	57.67	30	40.86	14	68.22	27	23.7
32EM	47.80	23	38.51	10	79.84	38	23.7
12.9PA	37.50	14	58.36	29	68.49	28	23.7
8EM	61.37	34	40.78	13	66.78	25	24.0
2.8VM	60.36	33	42.79	15	66.91	26	24.7

3.1NASS	49.30	26	56.58	27	64.39	23	25.3
2.3VM	34.90	12	58.63	31	79.17	37	26.7
9.3NASS	65.30	37	58.44	30	48.13	14	27.0
12.1PA	59.13	32	73.06	39	48.09	13	28.0
9.1PA	57.90	31	43.61	16	80.83	39	28.7
2.4PA	44.40	21	60.72	34	73.37	33	29.3
3.3VM	73.93	39	46.03	17	74.46	34	30.0
2.11BG	63.90	35	57.30	28	69.62	29	30.7
8.5PA	73.41	38	67.26	37	59.96	20	31.7
2.3BG	48.76	25	62.09	35	78.72	36	32.0
SEM	9.10		8.02		8.30		
LSD	34.10		30.04		31.01		
CV(%)	34.9		28.4		25.7		
Fishers' Level (%)	1		1		1		

SEM = Standard error mean; LSD = Least significant difference; C V (%) = Percentage coefficient of variance.

Table 8.4: Comparison of cashew anthracnose leaf incidence (%) on dwarf cashew progenies at Pebane, Mozambique, 2006-2008.

Year	2006		2007		2008		
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Overall Rank
22.3PA	*		*		*		
29EM	37.33	12	9.60	1	3.21	1	4.7
40EM	47	22	34.77	2	4.82	2	8.7
1.12PA	37.93	14	3.17	10	12.42	10	11.3
11.8PA	35.57	11	8.93	12	15.06	14	12.3
12.6PA	63.23	41	49.40	5	6.85	5	17.0
3.2VM	62.3	38	10.92	7	11.19	7	17.3
6.7NASS	22.4	1	6.00	26	21.73	26	17.7
2.5VM	43.8	18	5.50	18	19.19	18	18.0
55EM	69.17	48	2.87	3	5.75	3	18.0
1.20VM	57	33	55.60	11	13.97	11	18.3
31EM	44.47	19	0.43	19	19.62	19	19.0
30EM	25.73	4	5.83	15	18.55	39	19.3
19EM	37.87	13	2.47	23	21.3	23	19.7
9.4Nass	53.6	28	11.27	16	18.61	16	20.0
47EM	69.93	51	7.73	8	11.33	8	22.3

4.1AD	25.57	3	25.43	38	29.48	31	24.0
2.7Nass	77.77	59	28.07	9	12.40	4	24.0
12.1PA	31.5	7	8.73	4	6.60	62	24.3
41EM	54.77	29	28.57	22	21.04	22	24.3
37EM	33.87	9	9.93	32	25.62	32	24.3
2.3BG	69.77	49	30.53	13	16.13	13	25.0
2.3VM	83.93	63	16.20	6	7.93	6	25.0
48EM	55.43	30	11.10	24	21.58	24	26.0
1.18VM	34.87	10	0.00	25	21.72	48	27.7
1.4Nass	76.63	57	29.70	14	17.17	14	28.3
3.1Nass	57.37	34	44.43	34	25.87	18	28.7
7.10PA	71.73	53	13.23	17	18.74	17	29.0
53EM	68.47	46	30.20	21	20.90	21	29.3
20EM	23.7	2	0.00	45	37.02	45	30.7
38EM	33.57	8	16.47	36	28.83	50	31.3
2.4PA	39.3	15	27.70	40	31.11	40	31.7
25EM	45.17	21	16.83	37	29.15	37	31.7
3.11PA	72.8	56	8.73	20	20.08	20	32.0
42EM	57.77	35	0.80	43	34.93	18	32.0
57EM	31.03	6	4.40	47	27.60	47	33.3

60EM	58.23	36	12.13	33	25.75	33	34.0
12.9PA	43	17	6.63	44	36.61	44	35.0
1.3Nass	69.83	50	33.80	28	24.47	28	35.3
2.3PA	53	27	9.50	42	33.59	42	37.0
2.11BG	80.07	60	60.07	27	23.06	27	38.0
24EM	71.3	52	7.40	31	25.24	31	38.0
16.1BG	62.93	39	31.97	41	31.31	41	40.3
11.2PA	65.1	43	7.67	39	30.58	39	40.3
3.3VM	56	31	7.50	46	37.35	46	41.0
11.9PA	85.87	64	41.33	30	24.82	30	41.3
10.1Nass	77.1	58	43.97	35	28.58	35	42.7
6.7Nass	42.1	16	15.97	57	43.32	57	43.3
8.5PA	52.87	26	3.27	52	40.11	52	43.3
12.8PA	30.38	5	8.63	63	55.32	63	43.7
18EM	52.13	24	18.60	54	42.54	54	44.0
2.8VM	56.33	32	0.67	50	38.78	50	44.0
1.7VM	45.03	20	16.23	59	47.07	59	46.0
9.1PA	63.17	40	13.93	51	39.54	51	47.3
12.3PA	68.73	47	16.13	48	37.77	48	47.7
52EM	72	54	21.70	29	24.49	63	48.7

5.12PA	48.1	23	3.30	62	54.84	62	49.0
50EM	59.2	37	4.40	56	42.80	56	49.7
9.3Nass	72.5	55	29.50	49	38.60	49	51.0
39EM	65.17	44	17.92	55	42.58	55	51.3
43EM	67.37	45	56.10	58	44.05	58	53.7
12.2PA	64.27	42	33.30	61	49.45	61	54.7
11.7PA	*	66	9.50	53	40.59	53	57.3
5.9PA	83.2	61	26.80	60	47.81	60	60.3
16.1PA	52.33	25	*		*	*	
6.2PA	*		*		*	*	
9EM	83.47	62	23.70		8.27	*	
SEM	13.85		9.23		30.58		
LSD	38.75		25.84		30.58		
C V (%)	43.2		89.2		52.5		
Fishers' level (%)	5.0		5.0		1.0		

SEM = Standard error mean; LSD = Least significant difference; C V (%) = Percentage coefficient of variance; *Plants died due to stem borer.

Table 8.5: Comparison of cashew anthracnose leaf incidence (%) on common cashew progenies at Mocuba, Mozambique, 2006-2008

Year	2006		2007		2008		
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Overall Rank
NA7	26.56	8	6.06	1	22.52	1	3.3
MB77	27.57	10	14.78	3	24.49	3	3.3
MU2	24.86	6	18.96	5	45.74	12	5.3
103.82	20.83	4	34.68	14	45.60	11	7.7
103.81	9.80	1	19.72	7	65.83	23	9.7
Na100	40.43	21	17.28	4	38.52	6	10.3
MB76	40.40	20	27.12	9	30.15	4	10.3
1.5R	31.00	15	49.94	22	23.77	2	11.0
MU63	29.87	13	40.91	17	44.22	9	13.0
MS-2	23.13	5	42.39	18	57.13	18	13.0
02NASS	29.80	12	13.34	2	75.29	28	13.7
EBA70	37.77	17	21.60	8	55.71	17	14.0
MU106	41.67	22	28.78	12	42.94	8	14.0
NA96	33.03	16	34.61	13	45.94	13	14.0
NA98	29.43	11	35.40	15	52.93	16	14.0
PPE13	34.13	18	28.63	11	50.00	15	14.0
103.79	55.07	30	27.86	10	39.74	7	14.7
MU45	24.87	7	43.57	19	63.52	22	15.7
IM5	52.30	28	38.21	16	32.79	5	16.0
103.68	45.37	24	51.13	24	45.03	10	16.3
MU18	17.40	2	58.02	29	73.10	27	19.3
MU42	37.63	19	54.17	25	46.05	14	19.3
103.85	27.07	9	60.51	30	59.37	21	19.3
NA5	18.00	3	54.69	26	76.65	31	20.0
MU32	49.06	27	19.44	6	75.36	29	20.0
NC3	30.97	14	50.61	23	68.92	25	20.7
NC1	48.67	26	47.32	20	58.71	20	20.7
MB83	47.53	25	56.48	27	57.46	19	22.0

NA1001	55.40	31	48.20	21	68.52	24	23.7
MU1	42.40	23	69.28	33	75.50	30	25.3
MU3	72.33	33	57.55	28	69.74	26	28.7
MB75	54.76	29	65.01	32	78.82	32	29.0
IM1	65.06	32	61.35	31	84.58	33	31.0
SEM	7.63		8.34		3.75		
LSD	28.72		23.61		14.07		
CV(%)	35.7		36.7		11.9		
F.level(%)	1.0		5.0		1.0		

SEM = Standard error mean; LSD = Least significant difference; C V (%) = Percentage coefficient of variance.

Table 8.6: Comparison of cashew anthracnose leaf incidence (%) on common cashew progenies at Pebane, Mozambique, 2006-2008

Year	2006		2007		2008		
Identifier Mean		Rank	Mean	Rank	Mean	Rank	Overall Rank
1.5R	16.43	4	1.99	14	13.18	8	8.67
MCH-2	37.33	8	0	2	18.47	16	8.67
NA-7	50.94	3	5.72	18	13.6	10	10.33
NH-3	0	1	15.06	29	12.99	7	12.33
MS-6	63.64	30	1.85	13	6	2	15.00
MH-1	24.54	36	4.67	16	4.78	1	17.67
MS-5	25.28	32	1.58	12	20.42	18	20.67
MR-6	56.51	12	16.67	32	20.85	19	21.00

IN-4	47.57	11	8.66	20	24.36	33	21.33
MR-2	22.81	52	0	5	13.59	9	22.00
EBA-70	55.59	14	12.79	24	23.69	30	22.67
IN-1	19.02	29	2.91	15	23.15	26	23.33
MS-1	58.04	20	18.26	35	16.83	15	23.33
NNB-6	37.48	61	0	9	10.83	4	24.67
IM-5	16.67	7	50.77	66	10.12	3	25.33
MMC-4	4.11	47	0	4	23.2	27	26.00
IN-3	9.73	10	10.67	22	29.56	46	26.00
MB-76	0	43	5.57	17	21.63	20	26.67
NC-3	81.48	22	13.64	26	4.8	35	27.67
IN-2	48.65	25	21.98	39	21.72	21	28.33
02Nass	69.93	26	29.17	44	19.72	17	29.00
MMR-4	44.44	2	17.62	34	31.44	52	29.33
MMJ-3	55.21	15	16.67	33	28.35	42	30.00
MS-4	86.05	50	0	6	24.51	34	30.00
NC-1	39.03	24	31.3	45	22.36	22	30.33
IM-3	52.33	19	16.53	31	27.4	41	30.33
IM-2	19.33	79	0	1	14.84	12	30.67
NNT-4	93.85	21	0	10	36.06	62	31.00

Table 8.6 continued: Comparison of cashew anthracnose leaf incidence (%) on common cashew progenies at Pebane, Mozambique, 2006-2008

Year	2006		2007		2008		
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Overall Rank
NNT-2	29.53	37	18.81	36	24.24	28	33.67
NH-2	71.53	49	10.23	21	25.24	36	35.33
NH-4	42.2	76	0	8	22.38	23	35.67
MSC-1	48.9	73	0	7	24.19	31	37.00
NC-2	19.43	78	10.95	23	14.62	11	37.33
MMJ-2	41.5	17	25.29	42	32.18	53	37.33
MMR-6	58.86	16	33.33	48	30.15	49	37.67
103.85	91.6	28	60.9	73	16.12	14	38.33
NNT-6	32.08	66	7.78	19	24.33	32	39.00
MS-2	55.84	75	20.26	37	11.88	5	39.00
MO-1	89.72	38	16.46	30	30.26	50	39.33
NNB-1	3.85	51	14.83	27	28.88	43	40.33
ME-1	45.61	64	0	3	35.97	61	42.67
MMC-1	65.1	46	22.02	40	29.02	44	43.33

MH-2	80.31	9	55.05	68	32.7	54	43.67
MMR-5	25.52	48	40.37	60	22.57	24	44.00
NNB-2	42.6	42	33.63	51	26.63	39	44.00
IM1	6.48	13	37.86	55	39.38	66	44.67
PSP-2	86.06	33	33.33	50	30.63	51	44.67
MCR-5	40.37	60	59.46	71	11.9	6	45.67
MH-3	57.18	57	52.19	67	15.38	13	45.67
NNB-3	67.09	5	57.1	69	38.92	65	46.33
MCH-1	69.59	45	38.89	57	27.39	40	47.33
PPE-4	68.4	35	24.35	41	40.34	68	48.00
NC-4	51.68	23	39.79	59	38.58	63	48.33
NNB-5	81.47	27	65.33	76	29.09	45	49.33
PPE-15	61.94	18	38.04	56	46.43	75	49.67

Table 8.6 continued: Comparison of cashew anthracnose leaf incidence (%) on common cashew progenies at Pebane, Mozambique, 2006-2008.

Year	2006		2007		2008		
Identifier	Mean	Rank	Mean	Rank	Mean	Rank	Overall Rank
MR-9	90.45	6	50	64	70.2	80	50.00
MSC-2	96.44	69	13.27	25	33.73	57	50.33
IM-4	29.28	77	31.1	46	23.6	29	50.67
QM-1	87.57	40	61.22	74	25.73	38	50.67
MCL	97.03	34	33.33	47	45.66	73	51.33
NNT-3	44.06	68	14.96	28	34.33	59	51.67
MMR-3	5.48	63	39.49	58	25.34	37	52.67
NNT-5	55.98	80	0	11	39.59	67	52.67
MMP-2	75.47	53	21.67	38	40.67	69	53.33
PPE-13	57.12	59	37.33	54	29.98	48	53.67
MMJ-1	87.09	55	37.13	53	32.83	55	54.33
NM-2	78.93	44	47	62	35.26	60	55.33
PPE-18	27.85	65	27.66	43	38.9	64	57.33
PSP-1	67.48	72	79.75	77	23.04	25	58.00
CA13	81.18	39	50	65	45.88	74	59.33
MS-3	80.92	58	33.33	49	47.42	76	61.00
MR-1	26.13	41	62.25	75	43.3	70	62.00
PPE-14	39.88	70	41.79	61	33.17	56	62.33
MMP-1	69.61	56	49.16	63	44.3	71	63.33
NNT-1	92.41	31	100	80	65.44	79	63.33

MMR-2	63.11	67	35.85	52	44.36	72	63.67
PPE-16	65.38	62	60.28	72	33.79	58	64.00
QM-2	81.84	71	86.52	79	29.96	47	65.67
NM-2	98.16	54	84.77	78	59.42	78	70.00
MU-3	67.99	74	58.51	70	52.64	77	73.67
SEM	14.44		16.57		8.08		
LSD	40.33		61.13		29.78		
C V (%)	45.9		101.3		48.6		
Fishers' Level (%)	5		1		1		

SEM = Standard error mean; LSD = Least significant difference; C V (%) = Percentage coefficient of variance.

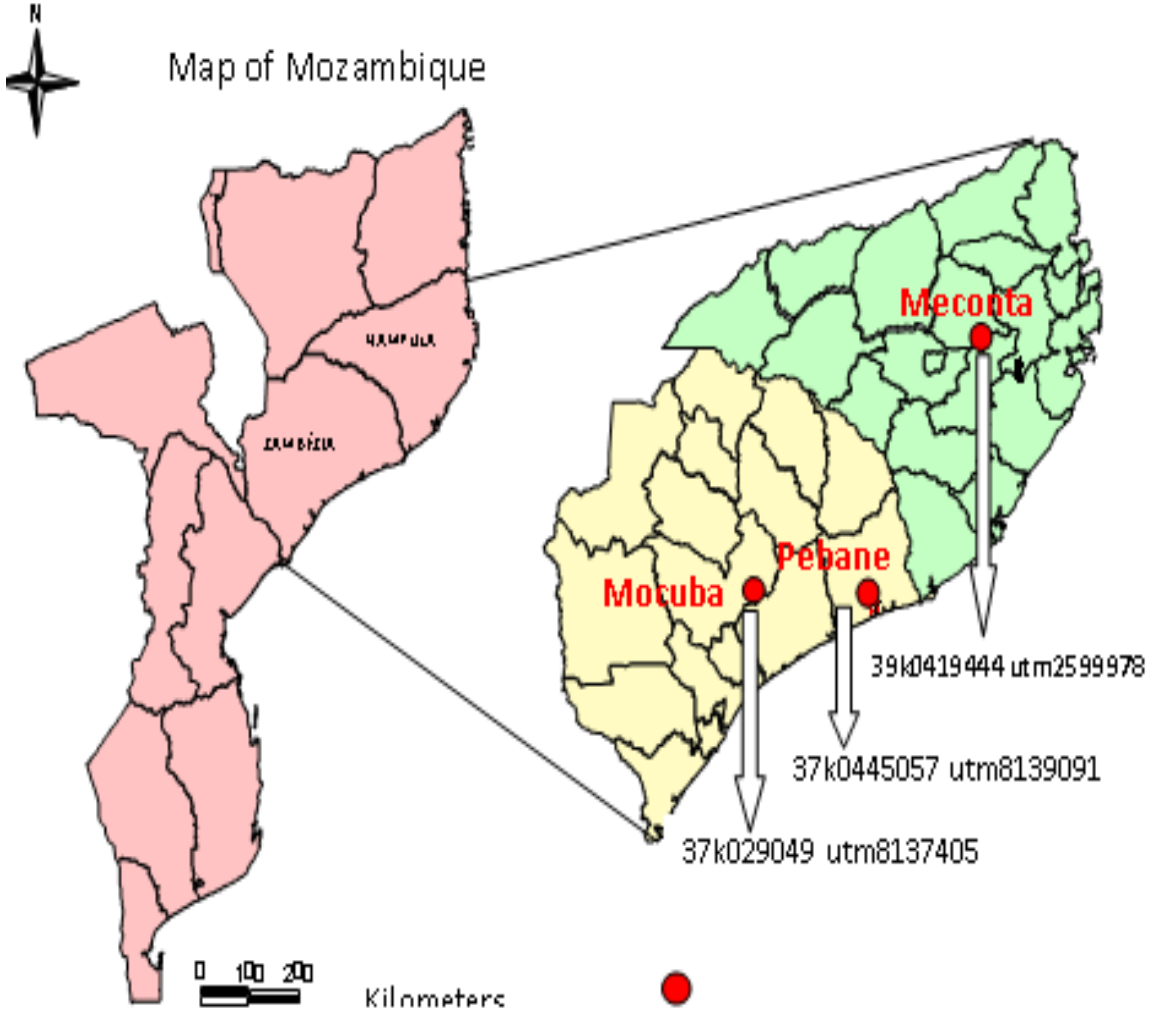


Figure 8.1: Anthracnose cashew genotypes screening trial sites in Mozambique, 2006-2008.

CHAPTER X: FINAL CONSIDERATIONS

Taxonomic identity of the anthracnose causal agent in Mozambique was confirmed to be *Colletotrichum gloeosporioides* Penz. by using the polymerase chain reaction technique. However, with development of new molecular tools, intra specific variations can be easily detected and used to guide management approaches. Symptoms distinctiveness in relation to other similar leaf diseases was established through pathogenicity test. But, pathogenic fungi isolated from cashew, occure as complexes. Therefore, future research should focus in understanding their interaction, impact on production so that integrated disease management approaches developed. The survey conducted in this study demonstrated that anthracnose disease is prevalent in all nurseries throughout the country but its incidence and severity varies with agro-ecological zones, nature of seedlings (grafted or rootstock) as well as the nursery hygienic conditions. In future, localization of cashew nurseries must be analysed considering the economics of disease management versus seedling transportation to the growing areas. Seedling spacing manipulation was found to be important as a disease control strategy in the nursery. Nursery infrastructures' design in areas of high disease pressure must include environment and seedling manipulation approaches that reduce disease development favorable conditions. Preventive sprayings against anthracnose are common practice in cashew nurseries. However in this study these applications were found to be justifiable only on seedlings with more than six leaves. By adopting seedling phenological age to initiate preventive fungicide applications on cashew seedlings, production costs can be reduced.

The causal agent of anthracnose was found to be hexaconazole sensitive both in the laboratory as well as in the field. The field trial demonstrated that this fungicide can also control powdery mildew at an economic frequency of at least 10 applications per season. In cashew, these applications are made within a period of three months followed by nine months before the commencement of the next crop season. Thus, the fungicide plan would allow enough time to minimize negative environmental impacts. Anthracnose disease epidemics were found to be associated with weekly mean rainfall of at least 1 mm. The disease incidence or disease severity on leaves varied from one location to another or one season to another but in all cases the relationship between the two parameters could be summarized in a single exponential equation. The equation was then recommended to simplify the disease assessment procedure and modeling of the disease development. Furthermore, the disease

scoring system developed in this study was used to screen a series of cashew cultivars for resistance to anthracnose disease. Some tolerant cultivars were identified among dwarf and common types.

Future work should focus on gene marking of tolerant genotypes as a way through for germplasm introductions. Continous characterization of *C. gloeosporioides* isolates and comparison by cross inoculation in a variety of cultivars and environmental conditions can be of practical value in development of local or global disease management strategies. The complexity of pathogens' interactions and similarities in reproduced symptoms on cashew suggests that describing inhabitants of cashew leaf surface and their relationships with pathogens and environment is required. A more holistic approache is necessary for understanding and successful management of cashew diseases in Mozambique.

The subjective aim of this thesis was to identify approaches that can be implemented in a developing country context: Proper diagnosis, safe and economical management of seedlings, adequate application of fungicides and selection of tolerant cultivars. These may be basic problems that most developed countries have overcome but in Mozambique these challenges remain a reality. Therefore they constituted sponsors' research priority for funding. In this regard the author considers this study to have successfully achieved the purpose of characterizing the pathogen, understanding the epidemiology of the disease and recommending some disease management strategies. In addition, we have explored opportunities for more extensive etiology studies and host/pathogen interactions under different agro-climatic conditions.

Academic contributions include, two articles published in an International journal (Appendices 12), other two articles published in edited proceedings of an international conference (Appendices 7 and 8) and an abstract published in edited proceedings of a national conference (Appendix 6). In addition, a poster (Appendix 2) and two handbooks were published (Appendices 9 and 10). In conclusion the present study provided a significant contribution towards reduction of cashew losses in Mozambique and has opened new avenues for future fruit tree research.

APPENDIX 1: The cashew map of Mozambique

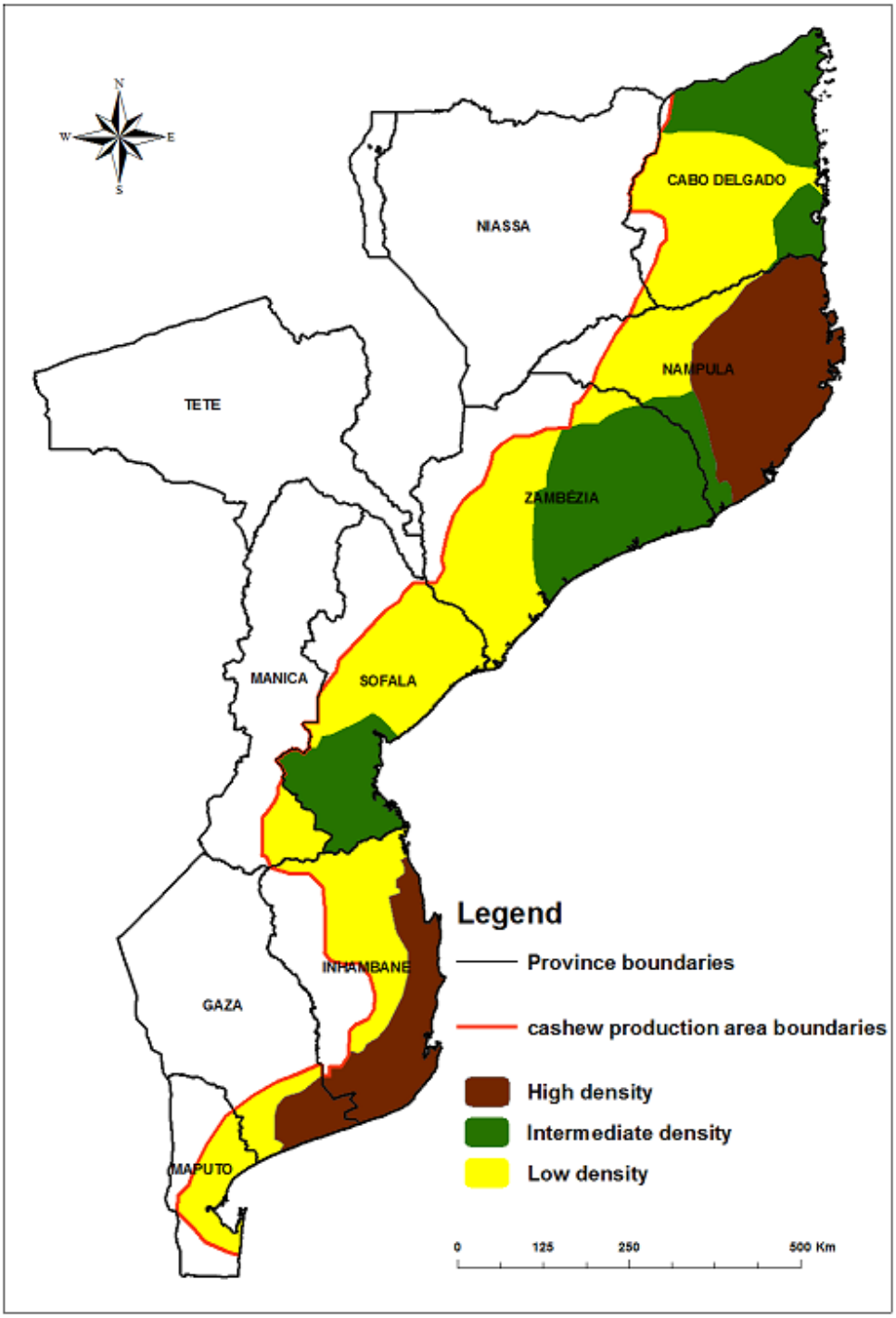


Figure 9: Cashew production area and plant density. Source: Ascenso J.C. and Pedroso, A.D., 1970.

APPENDIX 2: Calendar of events on cashew production in Mozambique

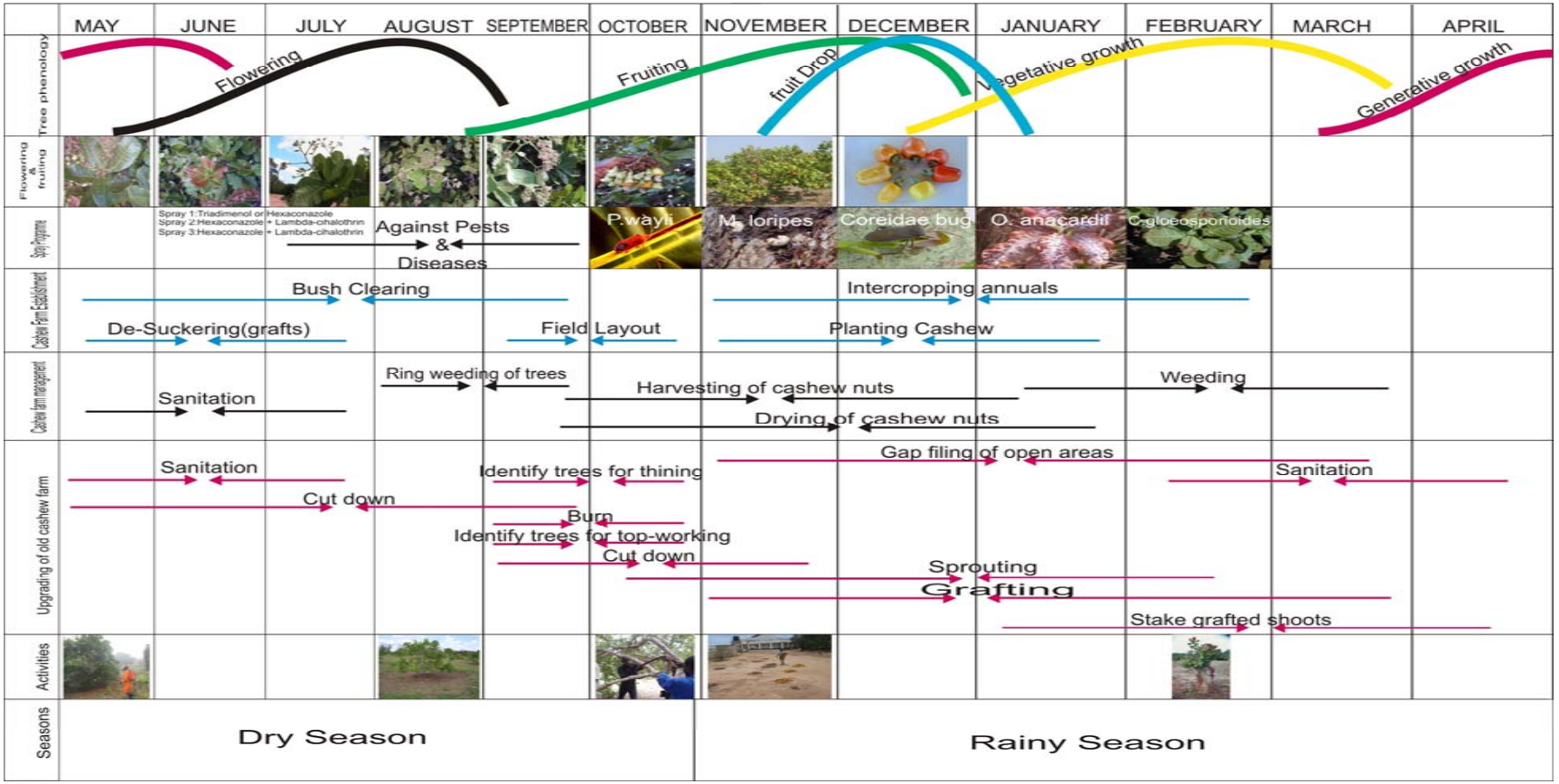


Figure 10: Cashew production cycle and main activities in Mozambique.

APPENDIX 3: Cashew phenological guide including visual key. Modified from Conticini, 1982.

Plate 8 a: Initial phenological stages

Third phase

The shoot has grown to 10 cm, the four most external leaves are 10-12 cm long, the next two are 6-8 cm in length while the internal leaves, which almost wrap the flower bud, are 2-3 cm long. The inflorescence length from the base bract (i.e. the ultimate small leaf not yet fully developed), is approximately 2-3 cm long. The reddish- brown color of the leaves is becoming attenuated and a yellow orange shade is beginning to appear at the bottom of the leaves.

Fourth phase

The earliest four leaves progressively lose their typical reddish brown color of the earlier stages and become pale green. The four younger leaves, formed later, still retain their native color, toning progressively in color from the petiole. The toning at this stage covers the first two to three veins. The four proximal leaves have reached their maximum size (between 13 - 17 cm long by 8-10 cm wide), whilst the four distal leaves are still growing. The inflorescence starts to open, the length from the inflorescence bract is approximately 8-10 cm, and the branches are 3-4 cm long and usually four to five in number.

Fifth phase

The rachis of the inflorescence grows longer reaching an average size of 12-14 cm from the bract. All the leaves are fully developed and the first four leaves have toned to a more or less intense green color, depending on the type of the cashew nut tree. The leaf area becomes thicker and coriaceous. The coloring of the four following leaves covers about 40-50% of the leaf area from the petiole, the inflorescence has opened further and the laterals (5 or 6) are 6-8 cm long.

Sixth phase

All the leaves are green and of a definitive size. The rachis of the inflorescence has grown longer to a length of 14-17 cm. The panicle has laterals fully spread and some are in anthesis.

Plate 8b: Intermediate phenological stages

Seventh phase

Approximately 50% of the flowers are open and the panicle is fully developed. The coriaceous leaves of the shoots are dark green in color.

Plate 8C: Advanced phenological stages

APPENDIX 4: Standard diagrams 1-4, showing panicles affected with powdery mildew disease (*Oidium anacardii* Noack)

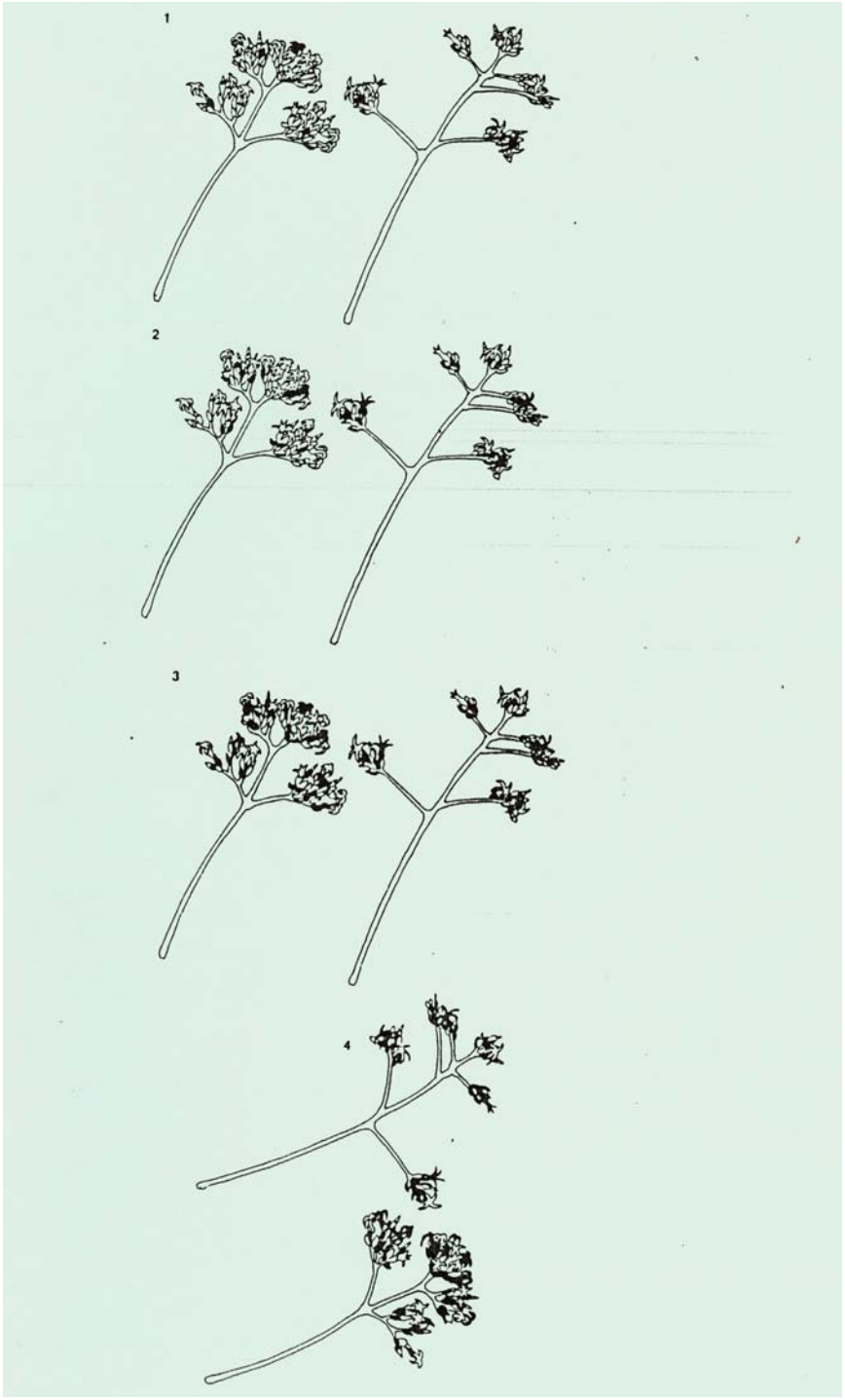


Figure 11: Powdery mildew infection levels. 0 = no infection; 1 =]0- 10%] flowers surface covered with actively sporulating mycelium, indicated by dark shading; 2 =]10 -25%] ; 3 =]25 -50%]; 4 =]50-75%]; 5=>]75-99%]; 6=>99%. Source: Nathaniels, 1996.

APPENDIX 5: Standard photos 1-5, showing cashew leaves affected with anthracnosis (*Colletotrichum gloeosporioides* Penz.)

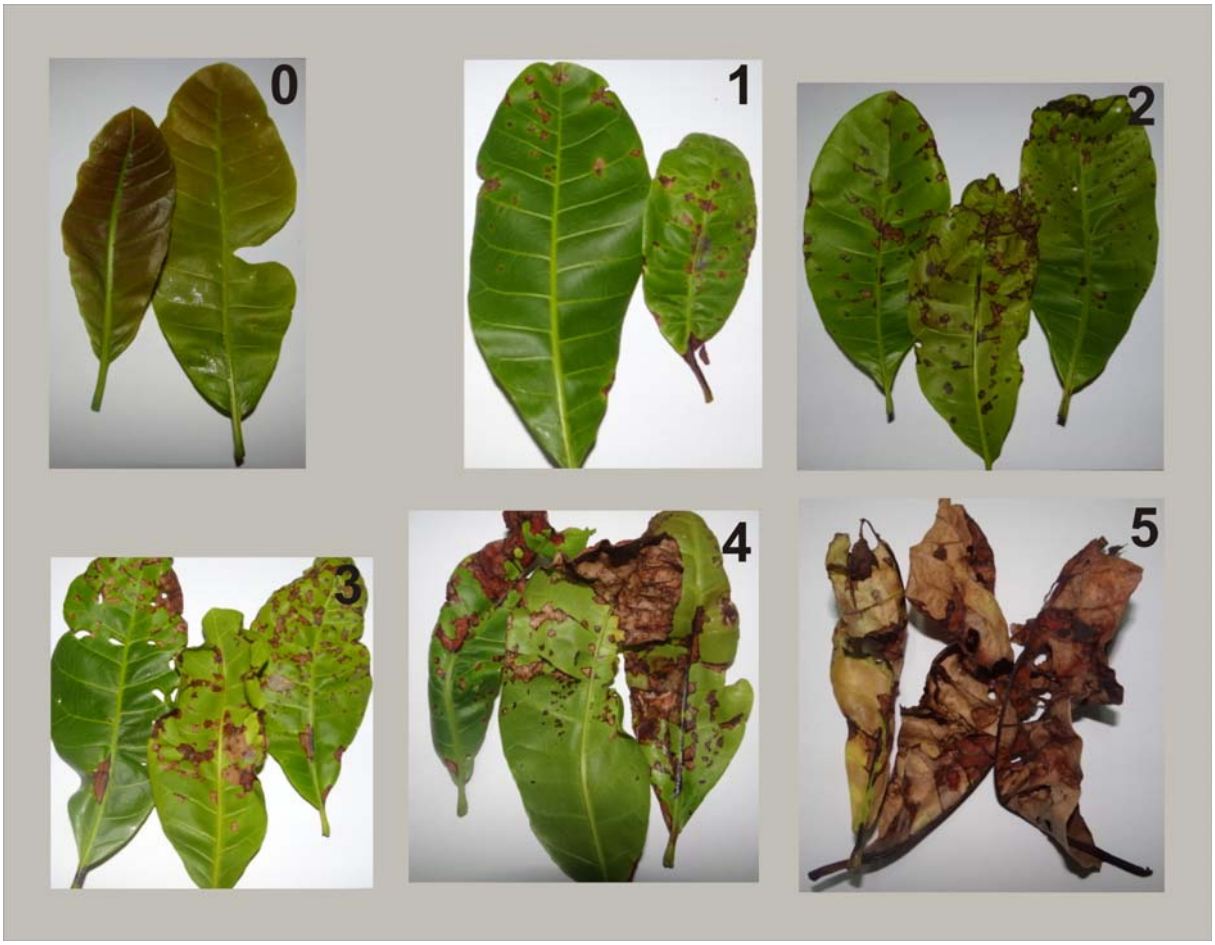


Plate 9: Anthracnose development stages: 1 =]0- 5%] leaf surface with necrosis, 2 =]5 -10%], 3 =]10 -25%], 4 =]25 -75%], 5=]75-99%], 6= >99-100%. Modified from Nathaniels, 1996.

APPENDIX 6: Conference abstract 1 (Portuguese)

Prevalência e maneio de doenças nos viveiros de cajueiro em Moçambique: O caso estudo da antracnose (*Colletotrichum gloeosporioides* Penz.)

Resumo

A propecção foi levada acabo em 32 viveiros de cajueiro seguindo o principio de localização por zonas agro-ecológicas. Os objectivos foram definidos como sendo: identificação das causas dos simtomas de folhas pretas reportados nas mudas, avaliar o nível de dano causado, identificar pontos fracos no maneio e formular recomendações apropriadas. O método adoptado para a prospecção consistiu num questionario previamente elaborado, avaliação de inicidência da doença ns mudas e colecta de amostra para posterior identificação do patógeno no laboratório. Os resultados indicaram que os sintomas observados estavam associados a *Colletotrichum gloeosporioides* Penz. agente causador da antracnose por vezes em associação com outros patógenos. A doença foi encontrada em todos os viveiros com média de maxima incidência de 6.22% nas mudas não enxertadas contra apenas 1.57% das mudas enxertadas. As zonas agro-ecologicas e épocas húmidas tiveram maior propensidade à epedemia com a maxima incidência de 7.60%. O período de Janeiro a Abril mostrou-se ser de maior prevalência da doença. Embora com média de incidência inferior a 1% a zona agro-ecologica R2, mostrou-se ser a mais problematica ao longo do ano. Em geral, práticas anti-fitossanitárias e sistemas de rega que promovem o surgimento de doenças associadas `a prevalência. A adopção do tratamento quimico apropiado, evitar dispersão de gotícolas de água durante a rega, desinfectar instrumentos e garfos, eliminar fontes de contaminação, etc. são algumas das medidas de maneio sugeridas.[4]

Palavras chaves: *cajueiro, antracnose, prospeccao e zonas agro-ecologicas*

[4] Abstract published in *Proceedings of the National Agriculture Conference, Maputo (2011).*

APPENDIX 7: Conference abstract 2

Status of the cashew subsector in Mozambique, current and future strategies

Abstract

This report summarizes the benefits of the current cashew scenario and provides an overview of challenges and perspectives for Mozambique. It reviews production trends, good agricultural practices, the research structure, research and extension linkages, the cashew nut processing industry and marketing of both raw nuts and kernels. Constraints and challenges are also discussed. Two major historical events, that is, the production and industrial crises, are highlighted as important episodes that impacted on the Mozambican cashew industry in the past and were reflected in both national cashew production and marketing. Consequently, production fluctuated from a peak of over 200,000 t in the early 1970s to about 18,000 t in the 1980s. However, institutional and operational changes i.e. close links between research and extension, through non-governmental organizations and government initiatives, resulted in a new growth phase that raised cashew production to over 100,000 t in 2004. A direct impact of the new impetus was the recovery of 6000 out of 10,000 job positions lost during the industrial crisis and more than US$20 million in foreign exchange earnings per year. Biological challenges identified include pests and diseases such as Helopeltis bugs, aphids, powdery mildew disease, anthracnose and the recently described leaf and nut blight. The use of polyclonal seeds combined with better understanding of germplasm and environment interactions evaluated through advanced approaches are the key aspects highlighted for a successful future in cashew production in Mozambique.[5]

Keywords: *cashew production trends, research and extension, Mozambique*

[5] Article published in *Proceedings of the Second International Conference, Kampala CAB International (2012:190-195.*

APPENDIX 8: Conference abstract 3

Leaf and fruit diseases of cashew (*Anacardium occidentale* L.) in Mozambique

Abstract

Leaf and fruit samples were taken from the most important cashew growing provinces of Mozambique in a year period to determine the presence and isolate already known pathogens at genus level. Symptoms associated with the pathogens in the field were described and pathogenicity tests conducted on detached cashew leaves and fruits. Multiple pathogens' simultaneous inoculations and symptoms development comparisons were made. Isolates growth rates on different media and sensitivity to different concentrations of the most commonly used fungicide on cashew was evaluated in vitro for *Pestalotia* sp., *Colletotrichum* sp., *Cryptosporiopsis* sp. and *Fusarium* sp. The results indicate that *Cryptosporiopsis* sp. is highly aggressive on host tissue but its growth on artificial media is lower than that of *Pestalotia* sp. Contrarily, *Pestalotia* sp., grows fast on artificial media but less aggressive on host tissue. For all the isolates, conidia and mycelia could not grow on liquid PDA modified with recommended concentrations of copper oxychloride, triadimenol, hexaconazole or trifloxystrobin fungicides. The study suggests that the success of holistic chemical control may depend on the deposition, frequency and economics of application rather than the mode of action of particular active ingredient.[6]

Keywords: *Cashew; Leaf and fruit diseases; Anthracnose; Fungicides.*

[6] Article published in *Proceedings of the Second International Conference, Kampala CAB International (2012):61-67.*

APPENDIX 9: Co-authored handbook 1

Diseases and Insect Pests of Cashew in Mozambique

Edited by

Liang Lihong, Zhang Zhongrun and Americo Uaciquete

Tropical Crops Genetic Resources Research Institute, CATAS, China

Instituto De Formento Do Caju（INCAJU）, Mozambique

March 2011

APPENDIX 10: Co-authored handbook 2

Cashew Cultivation and Processing

Edited by

Liang Lihong Americo Uaciquete

Tropical Crops Genetic Resources Institute, CATAS, China

Instituto De Fomento Do Caju (INCAJU), Mozambique

March 2011

APPENDIX 11: Survey questionnaire

Questionnaire on knowledge, prevalence and importance of cashew anthracnose in Mozambique

Plate 10: A: Pestalotia damage; B: Colletotrichum damage.

1. For the nursery personnel

1.1. Province……

1.2. District……..

1.3. Administrative Post……

1.4. Location……

1.5. Date……

1.6. Interviewer……

1.7. Respondent……

1.8. How many cashew seedlings (number) are in the nursery now?.....

1.9. For how long (number of days or months) the present seedlings have been maintained in the nursery?.....

1.10 How many (number) of the present seedlings have symptoms similar to those in the photo above?……

1.11. What is the ratio between diseased and healthy seedlings (that is X/Y ; x=diseased and y=total number of seedlings)……..

1.11.1. On grafted seedlings ……..…….

1.11.2. On non-grafted seedlings……..……...

1.12. Are the seedlings watered? (yes/no). How often (number of times per day or per week)…….……

1.13. Are the scions sprayed with fungicides before grafting?

Yes…….. No…….……

1.14. If yes, how?.....……

1.15. What is the effect of spraying? ……How is the spraying effect measured?…….……

1.16. Is there any of the years when you have no seedlings in the nursery?…… Which one (from…to…)?…….

1.17. Are the mother trees sprayed with chemicals? ……What is the chemical and rate of application ……. and frequency?…….. Aimed to control (pest and disease names) ……..

1.18. Are the seedlings in the nursery sprayed with any chemical?……...…….. Which one (name)?……. At which frequency?…….. and which rate of application?……...….

1.19. What is the actual damage of the symptoms observed on seedlings? (Signal the correct answers).

1.19.1. They only defoliate the seedlings……..…….……...…..….

1.19.2. They do kill the seedlings…….…….

1.19.3. They cause no problem to the seedlings……..……..…

1.20. When (period of months) are the symptoms observed? …….…….

1.21. When do (month) the appearance of new symptoms end?.....

1.22. Do nearby adult plants (up to 300 m) show similar symptoms or not?…….

2. For the scion personel

2.1. Province……

2.2. District……..

2.3. Administrative Post……..

2.4. Location……

2.5. Date……..

2.6. Interviewer……

2.7. Respondent……...

2.8. When preparing scions from mother trees, have you ever come across symptoms similar to those illustrated in the photos above?……..

2.9. Do such symptoms affect the quality of scions?……..……..…

2.10. If yes to 2.9, then how do you assess the damage?……....

2.11. What do you do to minimize the damage?……

2.12. Are the mother plants sprayed in order to control these symptoms? ……

2.13. If yes, what is the chemical, the rate and the frequency of application?……...……

2.14. Describe how the scions are cut from the mother tree, packed and transported to the nursery ……..……..……

2. 15. What basis (criteria) do you use to group the scions, e.g. by size, color, cultivar, quality, origin, etc. ……...……

……END…….…….