Epidemiology and control of powdery mildew (*Oidium anacardii* Noack) on cashew (*Anacardium occidentale* L.) in Mozambique

by

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CONTENTS

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SUMMARY

Epidemiology and control of powdery mildew (*Oidium anacardii* Noack) on cashew (*Anacardium occidentale* L.) in Mozambique

by

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For a successful and economical integrated control program aimed at a particular disease, pertinent information, regarding the environmental conditions prevailing in the growing area, the crop itself and the pathogen, must be available. Recently, the control of powdery mildew disease on cashew has moved from the use of non-systemic fungicides with a wide range of action, to highly specific systemic ones. Such a shift requires a more effective integrated control system, whereby tolerant varieties in combination with fungicide unaffected biocontrol agents are timely used to ensure disease control and reduce the hazards associated with excessive fungicide applications. The purpose of this study was to understand the relationship between the disease epidemic and some climatic factors over time. Appropriate periods for management interventions were determined. The cellular host reaction to infection by *Oidium anacardii* Noack was studied with a view to rapidly identify disease tolerant host types. Potential antagonists were isolated, screened and compared with commercial biocontrol products using *in vivo* techniques and chemical control programs were finally evaluated.

Electron microscopy elucidated that the powdery mildew tolerant cashew variety (H1) had a relatively higher consistency of cytoplasmic aggregates upon infection by *O. anacardii* when compared to the susceptible clone. Based on conidia and conidiophore morphology, conidial

germination and conidiogenesis processes observed indicated that *O. anacardii* belongs to the subgenus *Pseudoidium* (Y.S. Paul & J.N. Kapoor) comb. Et. Stat. Nov. (Holomorph *Erysiphe* Sect. *Erysiphe* U.Braun).

There was no direct relationship between the progress of the cashew powdery mildew epidemic and temperature, relative humidity or dew point over time. However, the epidemic did not start until conditions of average temperatures under the tree canopy were below 30ºC, relative humidity was 80% and dew point was above 15.

In vivo screening of 72 isolates, amongst them bacteria and fungi, from cashew leaves and florets showed that none were effective against *O. anacardii*, the causal agent of cashew powdery mildew. However, commercial antagonists, *Candida saitoana, Bacillus subtilis* and *B. licheniformis* significantly reduced the growth and branching of primary hyphae. One antagonist, *B. licheniformis,* was as effective as the commercial fungicide triadimenol 25% EC (Bayfidan).

Chemical fungicides were found to be effective against powdery mildew; however, the currently prevailing economic environment in Mozambique was found inappropriate for the use of expensive organic fungicides. Additional gain from the use of fungicides was found to be solely qualitative and thus did not represent a fair investment return ratio in terms of cashew nut prices and production costs. The use of integrated cashew management was finally recommended. Further studies should focus on development of integrated and cost effective disease management strategies.

CHAPTER 1

GENERAL INTRODUCTION

Cashew (*Anacardium occidentale* L.) is grown commercially mainly for their kernels (Ascenso, 1986). They are considered the third most sold nut (by mass) after hazelnuts and almonds (Masawe, 1993; Gunjate & Patwardhan, 1995; Rickson & Rickson, 1998). Besides the importance of its status as earner of valued foreign exchange, cashew also provides employment thereby stimulating the economy of developing countries, particularly in Asia, Africa and South and Central America (Rickson & Rickson, 1998). In general, every part of the tree is used, amongst others for medicinal purposes (Mitchell & Mori, 1987; Ferrão, 1995) and manufacturing of plastics, paints and varnishes (Mitchell & Mori, 1987). It is also used for clutch facing in the motorcar industry, insulating material (Behrens, 1996; Anon, 1997) and for preservation and protection of timber and crude fiber against insect and fungal attack (Bisanda, 1998).

Cashews are subject to various diseases that affect fruit, foliage, panicles, branches and seedlings resulting in major losses to the industry. These diseases include powdery mildew (*Oidium anacardii* Noack), anthracnose (*Colletotrichum gloeosporioides* Penzig et Saccardo in Penzig.), inflorescence blight (*Phomopsis anacardii* Early & Punit.) and damping-off of seedlings caused by *Fusarium* spp., *Pythium* spp. and *Phytophthora palmivora* Butler (Ohler, 1979; Milheiro & Evaristo, 1994; Nathaniels, 1994; Piteira, 1996). In Mozambique there were no recorded cashew diseases which had any major financial impact until the early 1960's (Silva, 1961). Anthracnose was later the major disease of cashew before the emergence of powdery mildew in 1973 (Milheiro & Evaristo, 1994; Anon, 1999). Today, powdery mildew is the most important disease (Nathaniels, 1996; Uaciquete, 1997; Topper *et al.*, 2000) causing losses of nut yield of between 50 and 70% (Milheiro & Evaristo, 1994). So far total host resistance (immunity) has not been found (Waller *et al.*, 1992; Martin *et al.*, 1997; Sijaona & Mansfield, 1998). Current management of the disease relies primarily on the application of either sulphur powder or water-based organic fungicides, tolerant cultivars (Masawe *et al.*, 1997; Smith *et al.*, 1997; Kasuga *et al.*, 1998; Topper *et al.*, 1998a, Topper *et al.*, 1998b) and cultural practices (Waller *et al.*, 1992; Masawe *et al.*, 1997; Shomari & Kennedy, 1999).

The adverse effects of synthetic chemical residues on human health and the environment (Korsten *et al.*, 1991; Rankovié, 1997; De Jager, 1999), development of pathogen resistance to certain chemicals (Sharma & Sankaran, 1988; Reuveni *et al.*, 1998), limited acceptance of fungicides for the export markets (Korsten *et al.*, 1991) and the desire to reduce pesticide levels on food crops (Reuveni *et al.*, 1998) have led to intensified research efforts worldwide to develop alternative control strategies (Sundheim, 1986; Reuveni *et al.*, 1997; Dik *et al.*, 1998; De Jager, 1999). To meet this goal, the utilisation of beneficial microorganisms to control diseases has been evaluated by various workers. Korsten *et al.* (1997) in three consecutive years, successfully used *Bacillus subtilis* integrated with copper oxychloride or benomyl to reduce severity of avocado black spot caused by *Pseudocercospora purpurea* (Cooke) Deighton. In addition, Dik *et al.* (1998) attained excellent control of cucumber powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht.:Fr.) Palacci) by weekly application of *Sporothrix flocculosa* (Traquair, Shaw & Jarvis). Furthermore, weekly applications of microconidial suspension of *Fusarium proliferatum* G6 (T. Matsushima) Nirenberg, teleomorph *Gibberella fugikuroi* (Sawada) Ito in Ito & K. Kimura, reduced downy mildew [*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni in Sacc.] on grape leaves and clusters (Falk *et al.*, 1996). Most of these studies focused on understanding and exploiting the natural microflora of the plant complexities involving the host, pathogen and bioagent (Sundheim, 1986) and how these are influenced by climatic conditions (Dik *et al.*, 1998).

This study was therefore conducted with the aim of understanding the mechanism of infection by the powdery mildew pathogen on various cashew cultivars with a view to identify sources of disease tolerance, understanding the epidemiology of the disease in southern Mozambique, isolating and screening some biocontrol agents as potential alternative disease control agents and evaluating different chemical products to manage the disease.

The work of this dissertation is a series of different topics organised into chapters. The literature review focuses on the taxonomy, origin and economic aspects of the crop as well as distribution and management strategies, including a conclusive analysis on gaps in knowledge. In Chapter 3, host / pathogen relationships and morphology of *O. anacardii* were investigated using scanning and transmission microscopy. The role of climatic parameters such as temperature, relative humidity and dew point in disease epidemic development was

investigated and presented in Chapter 4. In Chapter 5, potential antagonists were isolated and tested *in vivo* and *in vitro* against *O. anacardii* in comparison with commercial antagonists. In Chapter 6, field disease control programs with fungicides were tested. A general discussion with interpretation of results considering other references is presented in Chapter 7.

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CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Cashew vegetative growth is continuous during the first 15 years (Milheiro & Evaristo, 1994). 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 & Da Silva, 1995; Ferrão, 1995). 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 & Patwardhan, 1995; Behrens, 1996).

Powdery mildew caused by *Oidium anacardii* Noack severely attacks cashew at flower flush when the environment is favourable. The pathogen therefore infects immature leaves, shoots inflorescences, fruits (nuts) and false fruit (apple) (Anon, 1992; Milheiro & Evaristo, 1994). Protection against powdery mildew at generative growth phase is therefore essential.

This chapter deals with the host, the pathogen, its relationship with the host, disease epidemiology and management strategies.

CASHEW CROP AND ITS ECONOMIC IMPORTANCE

The first published illustration and description of the genus *Anacardium*, based on *A. occidentale* L., was provided by the French naturalist André Thevet in 1558 (Lopes *et al.*, 1993; Mitchell & Mori, 1987). The taxonomic position of the genus *Anacardium* was established by Bailey in 1942 (De Araujo & Da Silva, 1995). Thus, the genus *Anacardium* belongs to Division IV: Spermatophyta; Subdivision II: Angiospermae; Class II: Dicotyledoneae; Sub-class I: Archichlamideae; Order 39: Sapindales; Family: Anacardiaceae. The family Anacardiaceae consists of 60 to 74 genera and 400 to 600 species (Mitchell &

Mori, 1987). In addition to cashew, it contains a number of ornamental and fruit trees like sumach (*Rhus vernix* L.), pistachio (*Pistachia vera* L.), mango (*Mangifera indica* L.) and african plum (*Sclerocarya birrea* (A. Rich.) Aubr.) (De Araujo & Da Silva, 1995; Behrens, 1996).

Following classic or typological taxonomy, 21 species within the genus *Anacardium* were described. However, these were later reduced to only 10 species by using numerical systematics (De Araujo & Da Silva, 1995). No distinct cashew variety had been established until recently due to cross pollination, heterozygosity, and seed propagation (Lopes *et al.*, 1993; Gunjate & Patwardhan, 1995). However, four varieties based on apple colour, yield potential and size of the kernel have now been identified in India as Vengurla one, two, three and four (Gunjate & Patwardhan, 1995). In addition, two morphological types which markedly differ in size are recognised i.e. dwarf (small size) and common (giant in size) (Mitchell & Mori, 1987).

Cashew is a spreading, evergreen, perennial tree with a symmetrical umbelliform shape (Mitchell & Mori, 1987; Anon, 1992; Wild, 1994). The tree has a straight trunk and grows fast (Anon, 1992; Milheiro & Evaristo, 1994). The giant type can grow more than 20 m in height and 16 m in diameter over 25 years of age (Lopes *et al.*, 1993; Behrens, 1996). Cashews have an extensive root system consisting of one central taproot and a complex of extensive lateral roots (Lopes *et al.*, 1993; Milheiro & Evaristo, 1994). This complex root structure allows the plant to withstand long periods of drought (Anon, 1992). The leaves of the cashew 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 on both surfaces (Mitchell & Mori, 1987) and foliar nectaries present on leaf blades (Rickson & Rickson, 1998).

The inflorescence consists of a terminal panicle, 10-20 cm long, which is either sparse or congested (Mitchell & Mori, 1987; Duke, 1989). The panicle consists of both hermaphroditic and unisexual male flowers (Anon, 1992). The ratio between male and bisexual flowers varies from 2:1 to 200:1 (Milheiro & 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 registed (Ohler, 1979). However, productivity of individual trees is

determined not by number of fruits per panicle but by the number of panicles per tree (Milheiro & Evaristo, 1994) which varies from 100-400 depending more on the size of the tree than on genetic characteristics (Behrens, 1996).

Anacardium occidentale originated and was dispersed from tropical America (Lopes *et al.*, 1993; Milheiro & Evaristo, 1994; Ferrão, 1995). Many authors refer to north-east Brazil as being the center of origin for cashew since the most ancient description of cashew was found there (Lopes *et al.*, 1993; De Araujo & Da Silva, 1995). When the Portuguese landed in Brazil, cashew had already been disseminated throughout the coastal north and north eastern regions (Lopes *et al.*, 1993; Behrens, 1996). Today, it is estimated that more than 50 million trees still exist as remains of what has undoubtedly been a wider natural forest (Ferrão, 1995). Milheiro and Evaristo (1994) pointed out that the abundance and diversity of cashew species observed in this region constitute the physical evidence that cashew had its origin there. In addition, the Tupi Indians of the current north-eastern Brazil named the cashew tree "acaju". The original name was adopted into various languages and countries were it is cultivated today (Lopes *et al.*, 1993; Behrens, 1996). Finally, the diversity of delicacies traditionally made from the cashew reflect its long history in Brazil, the country of origin (De Araujo & Da Silva, 1995).

Cashew was introduced to most countries by the Portuguese (Lopes *et al.*, 1993). It is currently cultivated throughout the tropical world at low altitudes and commonly near the Atlantic and Indian oceans (Milheiro & Evaristo, 1994; Ferrão, 1995). The list of major producers include Guinea, Senegal, Nigeria, Angola, Congo Republic, Mozambique, Tanzania, Madagascar, Kenya, India, Sri-Lanka, Indonesia, Philippines, Malasia, Vietnam, Brazil, Panama and some islands in Australia (Milheiro & Evaristo, 1994; Ferrão, 1995; Gunjate & Patwardhan, 1995; Behrens, 1996; Anon, 1999).

An assessment of cashew world production based on processed nuts for the period 1951-1992 revealed three distinct phases (Milheiro & Evaristo, 1994). The first phase from 1951 to 1973 represented the expansion period for the industry. Nut production was characterised by the equation $Y = 94.415 + 23.306X$, where Y represents the nut production in tones and X represents the values 1, 2, 3, …n. One and "n" are the first and the last years of production in tonnage for the corresponding period respectively. The second phase extended from 1974 to 1980 and represented the declining phase, characterised by the equation $Y = 589.998$ -

26.321X. The third phase extended from 1981 to 1992, showing some production recovery represented by the equation Y= 427.644 + 5.195X.

Since 1965, Mozambique's production of cashew surpassed that of India (Hartman *et al.*, 1981) and remained so up to the 1970's when the country became the largest producer in the world with more than 200 000 tons of raw nuts (Wild, 1994; Uaciquete, 1997). However, when Behrens (1996) revised global trends of cashew nut production for the period 1989-1991, the largest producers were found to be India and Brazil, who shared 56% of the world market. Mozambique, with 7% of the world market, was the third largest producer of cashew nuts, which accounted for 50% of the country's agricultural exports. Following Mozambique were the newcomers Vietnam (6%) and Indonesia (5%). Tanzania, Nigeria and Guinea Bissau all being in the sixth position with 4% production, while Kenya, Sri-Lanka, Malaysia and Thailand represented only 2% each. All other producing countries combined had a total share of 6% of world production.

Current data indicates that India (60%) and Brazil (31%) are now the major exporters of kernels (Ascenso & Duncan, 1998). A striking aspect of the current global ranking is that production in Vietnam has increased to such an extent that it is now competing with Brazil as the largest producer, replacing Mozambique as a global player (Anon, 1998a). Current total world production of raw cashew nuts is estimated to be around 900 000 tons of which 400 000 tons per annum are from India. The next major producer, Brazil, accounts for a production of about 200 000 tons per annum (Nayar, 1998).

The main importers of cashew kernels are the United States of America (55%), Netherlands (10%), Germany (7%), Japan (5%) and the UK (5%) (Ascenso & Duncan, 1998). Other importing countries include Australia, Bulgaria, China, Hong Kong, India, the previous Soviet Union, France, Canada and Portugal (Ferrão, 1995; Anon., 1998b).

DISEASE EPIDEMIOLOGY

Causal agent: The anamorph known as *Oidium anacardii* (Moniliales: Erysiphaceae: Erysiphoideae) is a member of the genus *Oidium* Link (Ialongo, 1993) and subgenus *Pseudoidium* (Castellani & Casulii, 1981).

Origin and distribution: The first report of *Oidium anacardii* Noack on cashew is from São Paulo State, Brazil (Shomari, 1996; Martin *et al.*, 1997), where it is thought to have co-evolved with cashew (Waller *et al.*, 1992). The disease first appeared to be confined to this area until 1971, when Ponte and other workers found the disease in other areas of Brazil (Shomari, 1996). Subsequently, the disease has been reported to occur in India (Intini, 1987) although considered to be a minor problem (Shomari, 1996). In Africa, cashew powdery mildew was first officially reported in Tanzania in 1979 (Casulii, 1979) by which time it was already widely spread (Martin *et al.*, 1997). It is speculated that the disease was introduced to East Africa in infected planting material received from either Latin America or India (Intini, 1987). The disease has subsequently been reported from West African countries, Mozambique, El Salvador, Zambia, Kenya and Nigeria (Ohler, 1979; Shomari, 1996; Uaciquete, 1997; Mniu, 1998; Nayar, 1998). Finally, the pathogen is thought to be present in most cashew growing areas of the world although of minor importance (Shomari, 1996). In Mozambique, there were no recorded cashew diseases which had any major financial impact until the early 1960's (Silva, 1961). Due to its relative insignificance, powdery mildew was not even mentioned in a book by Carvalho and Mendes in 1958, describing major diseases of cultivated plants in Mozambique. Until the beginning of the 1970's, the disease was found to be sporadic (not recurrent) in nature (Milheiro & Evaristo, 1994). This suggests that the disease became noticeable only between 1960 and 1970 and became severe in 1973 (Anon, 1999).

Symptoms: The disease forms a whitish or light grey coverage consisting of mycelia, conidiophores and conidia on all parts affected (Anon., 1992). Affected flushes may fall early, floral buds wither and dry on the panicles. Apple and nut development can be completely interrupted (Anon, 1992; Waller *et al.*, 1992). On the leaves, the disease proliferates mostly on the upper part although it may also occur on the adaxial side (Piteira, 1996). They may lose the initial colour, turn purplish brown in patches and become malformed (Milheiro & Evaristo, 1994) or, manifest a reduced growth (Piteira, 1996). These organs dry immaturely or alternatively the disease ceases development when the infected tissue reaches maturity (Waller *et al.*, 1992; Shomari & Kennedy, 1999).

The pathogen attacks the flower stalk (pedicel), which develops into the cashew apple. Infection of cashew apples produces brownish-purple discolouration of the surface and restricts growth so that splitting may occur which allows entry of secondary fruit rotting

pathogens (Waller *et al.*, 1992). On the young nut itself, it can lead to stunting, corky rough surface blemishes (scarification), discolouration and, in severe cases, development of deep cracks in the cashew apple (Nathaniels, 1996). Scarring of the developing nut surface is the main symptom of nut infection (Waller *et al.*, 1992). This has been demonstrated to reduce the nut quality significantly by 5% as measured by shelling out-turn (Topper *et al.*, 2000).

Sporulation of the pathogen on the inflorescences is often more apparent than on other affected parts of the tree (Waller *et al.*, 1992), particularly soon after sunrise in a misty dry season (Uaciquete, 1997). Pathogen attack on the inflorescences has a more serious and direct influence on total production so that under certain conditions, trees produce little or no fruit at all (Anon., 1992; Waller *et al.*, 1992; Martin *et al.*, 1997).

Host Range: The powdery mildew pathogen is an obligate parasite and often host specific, at least to the genus level (Mount & Slesinski, 1971; Agrios, 1988). Due to its morphological resemblance, Waller *et al.* (1992) did not exclude the possibility that the powdery mildew pathogen (*Oidium mangiferae* Berth.) on mango may have extended its host range to include cashew, but so far no scientific studies have been conducted to explore this hypothesis.

Epidemiology: Cashew powdery mildew spores are wind-dispersed (Martin *et al.*, 1997). In a horizontal plane above ground, concentrations of *O. anacardii* conidia were found to be only 3.8% of the initial conidial concentrations at 180 m distance from the source. In a vertical position 16 m from the source, only 6.3% of the initial conidial concentration was present (Shomari, 1999 *personal communication*).

Conidia are known to germinate at relative humidities of 90 to 100% at an optimum temperature of 24-28°C (Castellani & Casulii, 1981; Martin *et al.*, 1997; Shomari & Kennedy, 1999). Shomari and Kennedy (1999) also studied the effect of temperature on conidial germination using a glass slide technique. They found that at 100% RH, germination was higher between 25 and 30°C than at 15°C, and that there was no germination at 35°C. They also reported that conidia suspended in water for 4 h resulted in only 1-2% germination. They also noted that water had no effect on appressorial formation.

The disease develops annually as an epidemic during the post rains or dry season (Waller *et al.*, 1992; Martin *et al.*, 1997), when a preponderance of young susceptible host tissue (principally inflorescence) and high humidity occur simultaneously (Waller *et al.*, 1992).

The disease starts from a small initial infection on highly susceptible young leaves within the canopy and on out-of-season inflorescences (Martin *et al.*, 1997). The peak of the epidemic coincides with the emergence of large numbers of susceptible inflorescences. Figure 2.1 illustrates the crop cycle during a year for the southern part of Mozambique. As the season progresses, mildew development declines as susceptible young tissues become less available and atmospheric humidity decreases (Waller *et al.*, 1992).

During the rainy season, the disease remains largely dormant as inactive (old) infections on mature shoots (Shomari & Kennedy, 1999). No evidence of cleistothecia formation has been found (Shomari, 1996; Shomari & Kennedy, 1999). At the dormant stage of the pathogen, it is difficult to detect the presence of the disease. However, actively sporulating lesions can readily be observed on sheltered and immature leafy shoots and occasionally on off-seasonal flowers throughout the year (Waller *et al.*, 1992). Thus, perennation of *O. anacardii* in the absence of the sexual stages is dependent on the interaction between host availability and the effect of the environment on the stages of the life cycle of the pathogen (Shomari & Kennedy, 1999) (Fig. 2.1).

Topper *et al.* (2000), working in Mozambique, summarised the annual life cycle of powdery mildew as follows:

- *Epidemic stage* - this is where the mildew is most noticeable attacking peripheral immature host tissues with inoculum coming from lower internal parts of the tree canopy (Fig. 2.2).

- *Decline stage* - when the powdery mildew cannot be found outside the canopy, but is found in minor branches under the canopy, on water shoots / suckers and leaves, where it is protected from excessive rain and heat.

- *Carry-over stage* - by the end of the rainy season, powdery mildew disease (PMD) is confined to some trees where there are susceptible tissues under the canopy. The

percentage of trees infected and the severity of infection at this stage varies according to the morphological characteristics of the trees. If they have a big closed canopy they will be more susceptible to PMD, since overcrowding / overlapping favours disease development. The climate also has an influence at the carry-over stage (end of the rain season), i.e. temperatures above 35°C during the rain season inhibits germination of conidia.

- *Outbreak stage* - spores released from the carry-over trees infect new tissue on the outside of the canopy and within one week are producing new spores (Fig. 2.1). From this source, further infection of recently emerged tissues (leaves and panicles) occurs. This stage finally results in an epidemic and the cycle starts all over again.

Host-pathogen interactions: The entry of fungal pathogens into healthy plant cells has been a subject of various studies based on light and electron microscopy (Aist & Bushnell, 1991). For powdery mildews, a number of morphologically identifiable stages of development such as spore germination, formation of appressoria, haustorial penetration, accumulation of cytoplasmic aggregates and formation of secondary hyphae or colony expansion were described (Yang & Ellingboe, 1972; Bushnell & Zeyen, 1976; Celio & Hausbeck, 1997; Leinhos *et al.*, 1997). The purposes for which the above processes are studied vary from a basic understanding of the mechanisms involved in host-pathogen interactions (McKeen & Rimmer, 1973; Bushnell & Zeyen, 1976) to histologically describing the activity of some fungicides (Leinhos *et al.*, 1997), determination of the effect of environmental conditions towards development of a potentially inexpensive disease control method (Sivapalan, 1993; Celio & Hausbeck, 1997) and identification of host-parasite incompatibility for resistance expression (Bushnell & Zeyen, 1976; Yang & Ellingboe, 1992).

The cashew powdery mildew pathogen anamorph was originally described by Noack in 1898 and revised by Castellani and Casulii in 1981, using light microscopy (Castellani & Casulii, 1981). The conidial germination process as affected by temperature, relative humidity and the influence of free water on leaf surface processes (pre-penetration and penetration) were investigated (Shomari & Kennedy, 1999). However, no details on host-parasite relationships, e.g. the infection process, were provided.

DISEASE MANAGEMENT STRATEGIES

Chemical control: Various researchers have demonstrated that fungicidal control of cashew powdery mildew can be achieved by application of wettable sulphur. This can result in significant yield increases from 1.4 to 11.6 kg (Waller *et al.*, 1992; Martin *et al.*, 1997). Control regimes started with fortnightly applications of 99% sulphur powder, 500 g/tree with seven sprays during flowering. In total, 200 kg of sulphur/ha is applied annually (Waller *et al.*, 1992). Later, reduced sulphur applications, by starting panicle dusting at the emergence stage, still provided effective powdery mildew control (Martin *et al.*, 1997). This even further reduced the applications to five at 250 g/tree with 14-day intervals for the second and 21-day intervals between the remaining three applications (Martin *et al.*, 1997; Kasuga *et al.*, 1998). According to Kasuga *et al.* (1998), the above recommendation is for dusting large trees (more than 10 m canopy diameter). The first application is timed according to action thresholds and results in a total of 1 250 g of 99% pure sulphur per tree per season.

The number of fungicide applications may be reduced even further by scouting for powdery mildew presence. For this purpose, dusting is not started until a minimum level of inflorescence emergence (20%) has occurred and there is a threshold level of 5% of inflorescences infected with mildew. Additionally, selectively targeting dusting on the best yielding trees can also reduce the amount of fungicides required (Martin *et al.*, 1997).

The reason why sulphur powder is currently recommended for mildew control throughout Tanzania is due to its comparative low cost and the fact that it does not require water for application. However, its sustained use has been shown to cause soil acidification with associated reduction in soil fertility and subsequent implications for long-term food security (Smith *et al.*, 1997; Kasuga *et al.*, 1998). Therefore, a number of water-based organic fungicides have been investigated as an alternative to sulphur (Topper *et al.*, 1998). Bio-efficacy trials demonstrated that fungicides, hexaconazole, E.C. 50 g a.i./l (Anvil), triadimenol, E.C. 250 g a.i./l (Bayfidan) and penconazole E.C. 100 g a.i./l (Topas) can give superior mildew control on cashew compared to sulphur (Smith *et al.*, 1997). The recommended rate for the use of Bayfidan and Anvil is 10 to 15 ml per tree (depending on tree size), applied in up to one l of water per tree (Kasuga *et al.*, 1998).

The economic yield for chemical disease control is estimated to be 4 - 6 kg of nuts per tree depending on the type and price of fungicide and the prevailing farm-gate price of cashew (Kasuga *et al.*, 1998). Trials carried out in Mozambique show that in plots treated with either Anvil or Bayfidan, average yields were greater than 20 kg/tree (Topper *et al.*, 2000). This represents a good economic return of US$12-34/tree/season. The corresponding currency income was only US$6.82/tree on sulphur plots. However this return was higher than that from the control plots which was only US$2.14/tree.

Biological control: There have been many attempts over a considerable period of time to obtain control of fungal pathogens by the use of antagonistic bacteria, yeasts or fungi (Skidmore, 1976; Pusey, 1989; Pruvost & Luisetti, 1991; Korsten *et al.*, 1992). Biological control of various powdery mildew fungi has been no exception (Rankovié, 1997; Dik *et al.*, 1998). The genus *Ampelomyces* is a well known hyperparasite and is widely distributed on powdery mildews (Sundheim, 1986; Rankovié, 1997). A number of other powdery mildew biocontrol agents such as *Telletiopsis* spp., *Cladosporium* spp. and *Acremonium alternatum* (Linc) Fr. have been reported (Sundheim, 1986), but results from practical field trials are less numerous (Tronsmo, 1992). The fungus *Ampelomyces quisqualis* Ces. and *Verticillium lecanii* (Zimm.) Viegas have been commercially formulated and registered as commercial products AQ10 and Mycotal respectively (Romero *et al.*, 2000). Effective biological control of mango powdery mildew (*Oidium mangiferae* Berth.) has been demonstrated (Korsten *et al.*, 1992). Furthermore, the yeast-like fungus *Sporothrix flocculosa* (Traquair, Shaw & Jarvis). has been demonstrated to be effective against both rose (*Sphaerotheca pannosa* (Wallr.:Fr.) Lév.) and cucumber powdery mildew (*Sphaerotheca fuliginea* (Schlecht.: Fr) Palacci) (Dik *et al.*, 1998). To our knowledge, there is not much information available regarding biological control of cashew powdery mildew. Casulii (1979) however, pointed out that the frequent occurrence of the hyperparasitic fungus *Cicinnobolus cesatii* De Bary, in association with cashew powdery mildew, could in future constitute a basis for biological control of the disease.

Control through genetically improved cashew planting material: Genetically improved cashew planting material currently available has been selected for its ability to produce cashew nuts of high quality in the absence of effective powdery mildew control measures (Martin *et al.*, 1997; Prasad *et al.*, 2000). The occurrence of partial resistance, amongst local

populations and collections of cashew germplasm, makes selection and breeding an obvious choice for long-term disease control (Waller *et al.*, 1992; Prasad *et al.*, 2000).

Breeding procedure for identifying local individual trees, with good performance against biotic and abiotic stresses has been practised in Mozambique and Tanzania over a number of years (Milheiro & Evaristo, 1994; Masawe *et al.*, 1998). Unfortunately, in Tanzania, a careful evaluation of the locally selected mother-trees, which appeared to be resistant to powdery mildew, resulted in these genotypes performing disappointingly. None of the selected genotypes yielded significantly more than the standard control (Caligari, 1997). Since 1986, outstanding local collections have been made at Naliendele in the search for new sources of germplasm. In addition, new introductions to Tanzania are being made from Senegal, Malawi, Zambia, Brazil, the Cook Islands and Mozambique. Selections made from the above mentioned collections are currently being used in the cashew breeding program started in 1990 in Tanzania (Martin *et al.*, 1997).

Some cashew clones tend to escape the mildew epidemic by flowering earlier or later than normal, representing apparent resistance. Late flowering is not considered a useful characteristic because the quality of the nuts is likely to be poor. These nuts will be harvested after the onset of the rainy season when drying could be difficult and when farmers are busy planting annual crops (Martin *et al.*, 1997).

The use of random amplified polymorphic DNA (RAPD) markers has previously been shown to be linked to agronomically important characters in a number of plants (Mneney *et al.*, 1998). Therefore, the use of RAPD-profiling of cashew cultivars, may soon become a procedure for large-scale screening of populations for assessing genetic diversity and to develop a marker-assisted breeding system for cashew.

Cultural control: No resting stage of the pathogen has been found and the disease perennates through the wet season mostly on water-shoots within the tree canopy (Martin *et al.*, 1997; Shomari & Kennedy, 1999; Topper *et al.*, 2000). Removal of inoculum before flowering delays the onset of mildew (Martin *et al.*, 1997; Kasuga *et al.*, 1998; Topper *et al.*, 2000). Therefore, regular sanitation of orchards could contribute to disease control (Maddison *et al.*, 1998). In addition, sanitation appears to be effective if a sufficiently large area is treated, combined with environmental conditions unfavourable for pathogen growth

(Martin *et al.*, 1997). Selective thinning of non-productive or overcrowded trees creates less favourable environmental conditions for mildew (Martin *et al.*, 1997). This is particularly true if thinning is applied towards the end of the dry season when farmers are cleaning their farms for annual cultivation (Martin *et al.*, 1998). Selective thinning can be followed by either filling gaps with improved cashew materials, top-working or inter-cropping with food crops (Kasuga *et al.*, 1998).

CONCLUSION

Powdery mildew disease is an economically important disease throughout east Africa. The pathogen, disease symptoms, epidemiology and management strategies have been seriously investigated for over two decades in Tanzania. However, the effect of geographic environmental variations on cashew powdery mildew epidemics, host-pathogen interactions with a view to identification of host resistance for powdery mildew as well as microbial biological control of the disease have not been explored.

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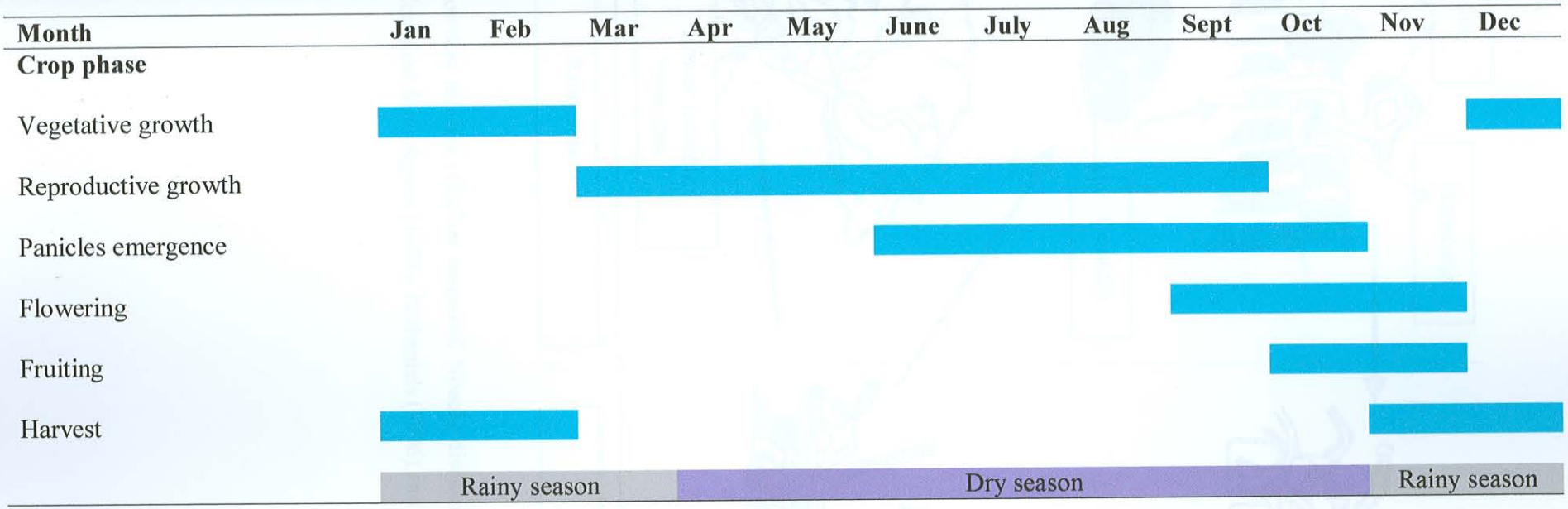


Figure 2.1 Cashew (*Anacardium occidentale* L.) crop cycle in Southern Mozambique (Milheiro & Evaristo, 1994).

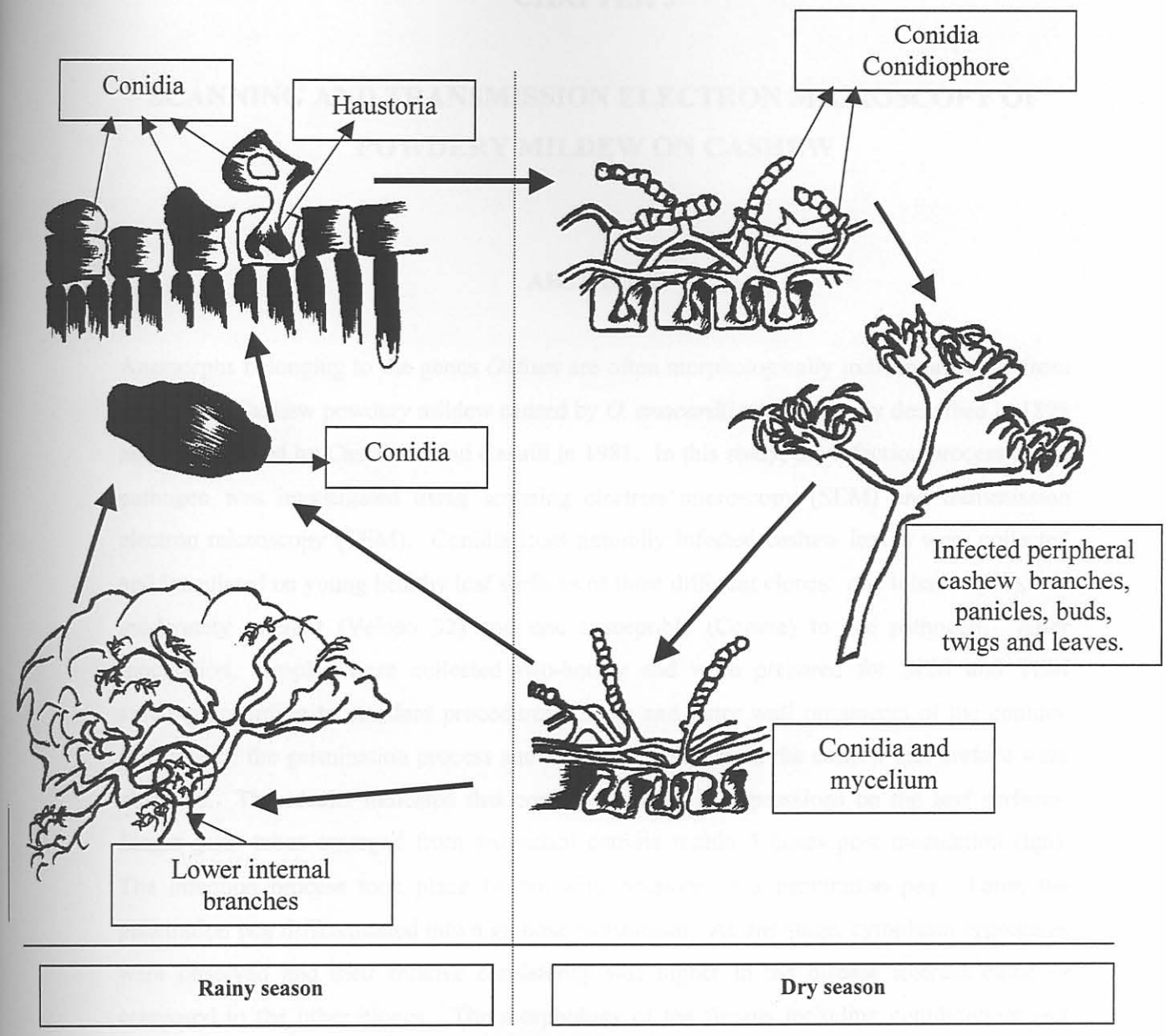


Figure 2.2 Powdery mildew (*Oidium anacardii* Noack) disease cycle on cashew (*Anacardium occidentale* L.). Compiled from Agrios (1988); Nathaniels (1996) and Shomari & Kennedy, (1999).

CHAPTER 3

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF POWDERY MILDEW ON CASHEW

ABSTRACT

Anamorphs belonging to the genus *Oidium* are often morphologically indistinguishable from each other. Cashew powdery mildew caused by *O. anacardii* was originally described in 1898 and later revised by Castellani and Casulii in 1981. In this study, the infection process of the pathogen was investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Conidia from naturally infected cashew leaves were collected and inoculated on young healthy leaf surfaces of three different clones: one tolerant (H1), one moderately tolerant (Veloso 32) and one susceptible (Corane) to the pathogen. After inoculation, samples were collected two-hourly and were prepared for SEM and TEM viewing according to standard procedures. Septa and outer wall ornaments of the conidia, sequence of the germination process and conidial formation on the cashew leaf surface were observed. The results indicated that conidia occurred in depressions on the leaf surfaces. Single germ-tubes emerged from individual conidia within 4 hours post inoculation (hpi). The infection process took place 14 hpi with emission of a penetration peg. Later, the penetration peg differentiated into a globose haustorium. At this stage, cytoplasm aggregates were observed and their relative consistency was higher in the disease tolerant clone as compared to the other clones. The morphology of the fungus including conidiophore and conidia, was observed and analysed using Cook's key. Based on the morphological observations we supported the taxonomic positioning of *O. anacardii* as a member of the subgenus *Pseudoidium*. The mechanism of infection was observed and no obvious host structural differentiation as response to the pathogen could be detected.

INTRODUCTION

Various factors have been ascribed to the decline in cashew (*Anacardium occidentale* L.) production in Mozambique (Uaciquete, 1997; Neto & Caligari, 1998). However, a critical analysis revealed that the lack of varieties and hybrids capable of producing more significant and durable levels of resistance to biotic stresses is one of the major causes of decline in production (Prasad, 1998).

The occurrence of apparent partial resistance in local populations and collections of cashew germplasm has been reported (Waller *et al.*, 1992). This is sometimes termed slow mildewing and is thought to be polygenically inherited and independent of known major race-specific genes for resistance (Aist & Bushnell, 1991). So far there is no evidence that major genes exist that give desirable levels of powdery mildew tolerance or resistance in cashew (Caligari, 1997).

Three approaches (Sijaona & Mansfield, 1998) have been used to study variations in cashew susceptibility or resistance due to mildew infection, covering both laboratory and field observations. Organs such as detached leaves, flowers and panicles including entire seedlings have been used in screening for tolerance (Sijaona & Mansfield, 1998). However, none of the previous studies illustrated the mechanism of interaction involved in host- pathogen relationships.

Anamorphs belonging to the genus *Oidium* Link are often morphologically indistinguishable from each other (Ialongo, 1993). Therefore, the taxonomy and identification of powdery mildews are based largely on the characteristics of the teleomorph (Cook *et al.*, 1997). However, the habit of using the name of the teleomorph to indicate the anamorphs resulted in limited studies of the latter (Ialongo, 1993). Identification based on teleomorphs are also a problem when the powdery mildew pathogen widens its host range or increases its ecological area of occurrence (Cook *et al.*, 1997). This is because the teleomorphic stage may not be found for some time or may not be produced at all. Furthermore, accurate identification is essential in plant quarantine and in locating the origin or source of inoculum (Cook *et al.*, 1997).

Determining the source of inoculum, as an element of disease dynamics, is of importance in developing a management strategy (Maddison *et al.*, 1998). In trying to do so, Waller *et al.* (1992), described the possibility that the powdery mildew of mango extended its host range to include cashew. Maddison *et al.* (1997) suggested that the cashew powdery mildew anamorph may also attack a wild fabaceae plant, *Julbernardia globiflora* (Benth.) Troupin, in Tanzania. However, no further clarification was provided on pathogen specificity and the potential of this miombo vegetation as an active reservoir or source of inoculum. Thus, a clear understanding of the pathogen's distinctiveness is required. The present study was designed to 1) provide further information on the characteristics of the cashew powdery mildew anamorph using scanning electron microscopy (SEM) to address the issue of identity and; 2) to describe the mechanism of infection on different cashew clones known to express different levels of susceptibility or tolerance to the disease using transmission electron microscopy (TEM).

MATERIALS AND METHODS

Plant inoculation

Vegetatively propagated and rain fed cashew trees over 30 years old grown at Ricatla cashew research station, Maputo, were used in this experiment. These trees were not used for infection studies due to a lack of appropriate facilities in Mozambique for an in-house study. In addition, South African quarantine authorities did not grant a permit for cashew plants to be brought into South Africa for experimental purposes. Since mature tissues are not susceptible to the disease (Sijaona *et al.*, 2001), only symptomless emerging twigs were used. One day prior to inoculation, conidia were shaken from infected cashew leaves to encourage new production before collection of inoculum. Young leaves from individual twigs were randomly selected and surface sterilised by dipping them up to the fifth leaf into 70% v/v ethanol for 2 min. The twigs with leaves were then rinsed twice in sterile distilled water and air dried before being inoculated with prepared inoculum. A dry brush was used to gently harvest spores and transfer them onto the new target young leaves.

Targeted leaves were inoculated on the upper surface over the whole lamina just after sunrise (06h00). Inoculated leaves were immediately covered in plastic bags previously moistened with sterile distilled-water. Three compatible combinations of cashew and powdery mildew inoculum were tested on clone H1 representing tolerant material, Veloso 32 as intermediate and Corane 2 as susceptible. Naturally infected and non-infected controls were included.

Samples consisting of 0.5 x 0.5 cm pieces cut from any site on the inoculated leaf lamina, were collected two-hourly until late at night (22h00). At each sampling time, individual samples were fixed in 2.5% glutaraldehyde, buffered with 0.075 M phosphate buffer. Samples were maintained at 6°C until transported to the laboratory for processing.

Scanning electron microscopy

After rinsing three times in 0.075 M phosphate buffer (pH = 7.4) for 15 min, samples were post-fixed in 1% unbuffered osmium tetroxide for 30 min at room temperature (Glauert, 1975). All material was successively washed three times in distilled water and then dehydrated in an ascending ethanol series (50%, 70%, 90% and three times 100%). Samples were critical point-dried in a Bio-rad E3000 critical point drier (Polaron Equipment Ltd, Hertfordshire, England) and mounted on SEM stubs. Specimens were coated with gold in a Polaron E5200 Sputter Coater (Polaron Equipment Ltd, Waterford, England) before examination with a JEOL JSM840 (JEOL LTD, Tokyo) SEM, operated at 5 kV.

Transmission electron microscopy

Samples were prepared for TEM as described up to the 100% ethanol dehydration step and were then infiltrated with quetol resin at 33% for 1 h, 66% for 1 h and twice in 100% for 18 h and 3 h respectively (Van der Merwe & Coetzee, 1992). Samples were polymerised at 60°C for 24 h. Gold sections were cut on an ultramicrotome (Ultracut E, Reichert-Jung, C Reichert, Vienna). Sections were stained with uranyl acetate for 30 min followed by lead citrate for 3 min. Sections were then viewed in a Philips 301 TEM (Philips, Eindhoven) operated at 60 kV.

RESULTS

Conidial germination and appressorium development

Conidia were generally clustered in depressions on the leaf surface (Fig. 3.1a). In certain cases, conidia were observed slightly embedded in the waxy layer of the upper leaf surface (Fig. 3.1b).

A single-germ tube emerged from individual conidia within 4 h after inoculation (Fig. 3.1c) and elongated (Fig. 3.1d). When it reached about 40 nm long, it swelled, especially at the apex, producing a long (10 nm), lobed structure (Fig. 3.1e), which is the primary

appressorium formed within 8 hpi. At 10 hpi, the primary appressorium differentiated dichotomously on the host epidermis in all material studied (Fig. 3.1f). No obvious differences between clones could be detected up to this stage.

Infection process

The infection process took place within 14 hpi. A penetration peg emerged and penetrated perpendicularly through the epidermal cell wall of the host (Fig. 3.2a). The host cell wall appeared visually thicker than that of adjacent uninfected cells and the cytoplasm beneath the penetration site aggregated (Fig. 3.2a). The penetration peg appeared narrowed at the penetration site and then expanded into a globular structure once inside the cytoplasm (Fig. 3.2b). The cytoplasm aggregates as well as the thickening of the cell wall were universal phenomena in all three clones. However, cytoplasm density was comparatively higher in clone H1 (as revealed by the concentration of stains) than other clones (Fig. 3.2b as compared to Fig. 3.2a). Otherwise, no obvious differences between clones could be observed.

Conidiophore and conidial development

A mature colony consisted of a matrix of abundant filamentous, septate and whitish hyphae. Conidiophore initiation was observed at 72 hpi as small globose swellings on the hyphae of mature colonies on infected leaves (Fig. 3.3a). The swellings differentiated into a typical conidiophore with a straight basal cell (Fig. 3.3b) and mature conidium with a distinct septum at the apex (Fig.3.3c). This in turn developed into two to three cells separated by septa (Fig. 3.3d). The distal cell matured and was released from the chain at the apical septum (Fig. 3.3d).

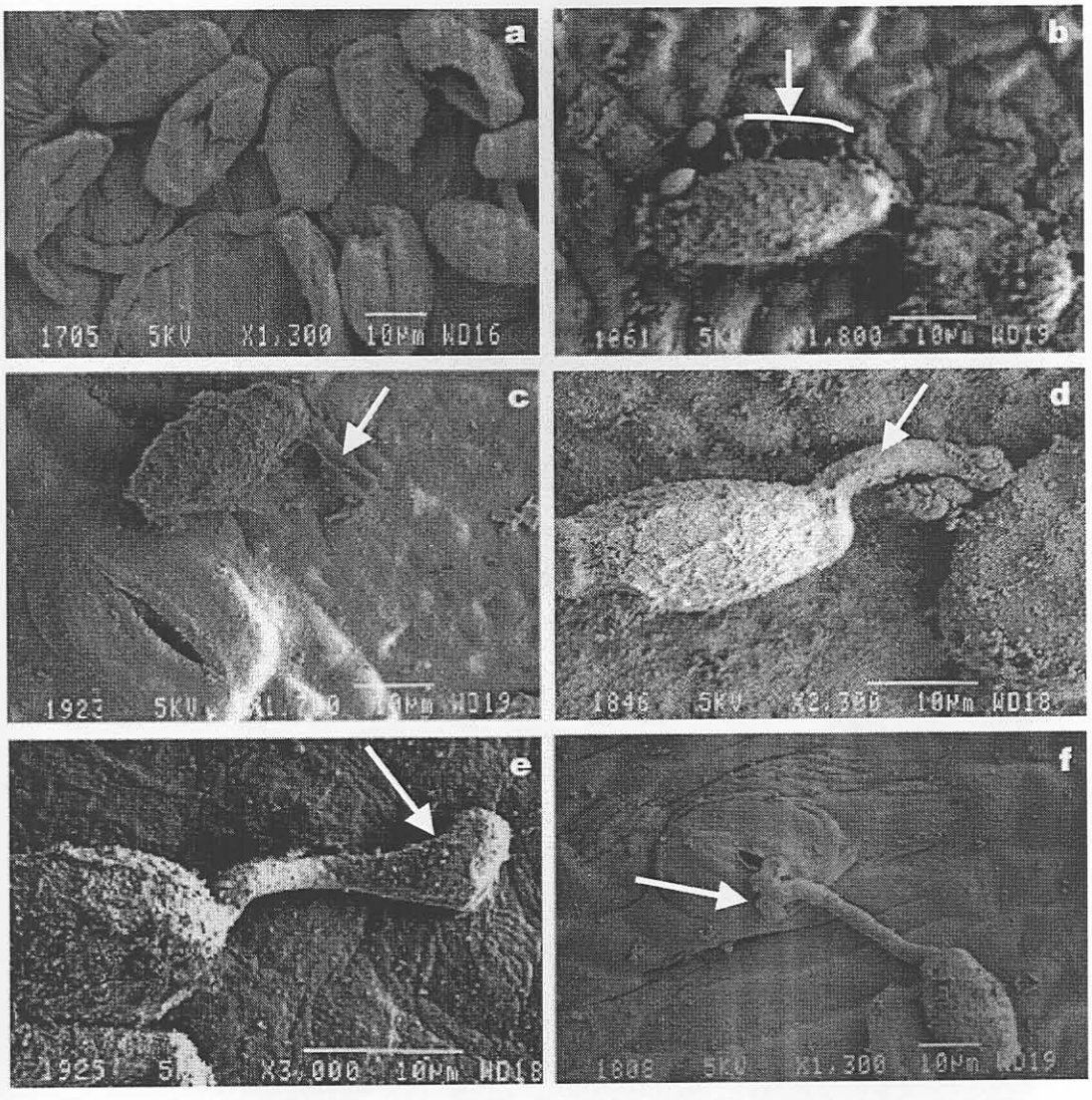
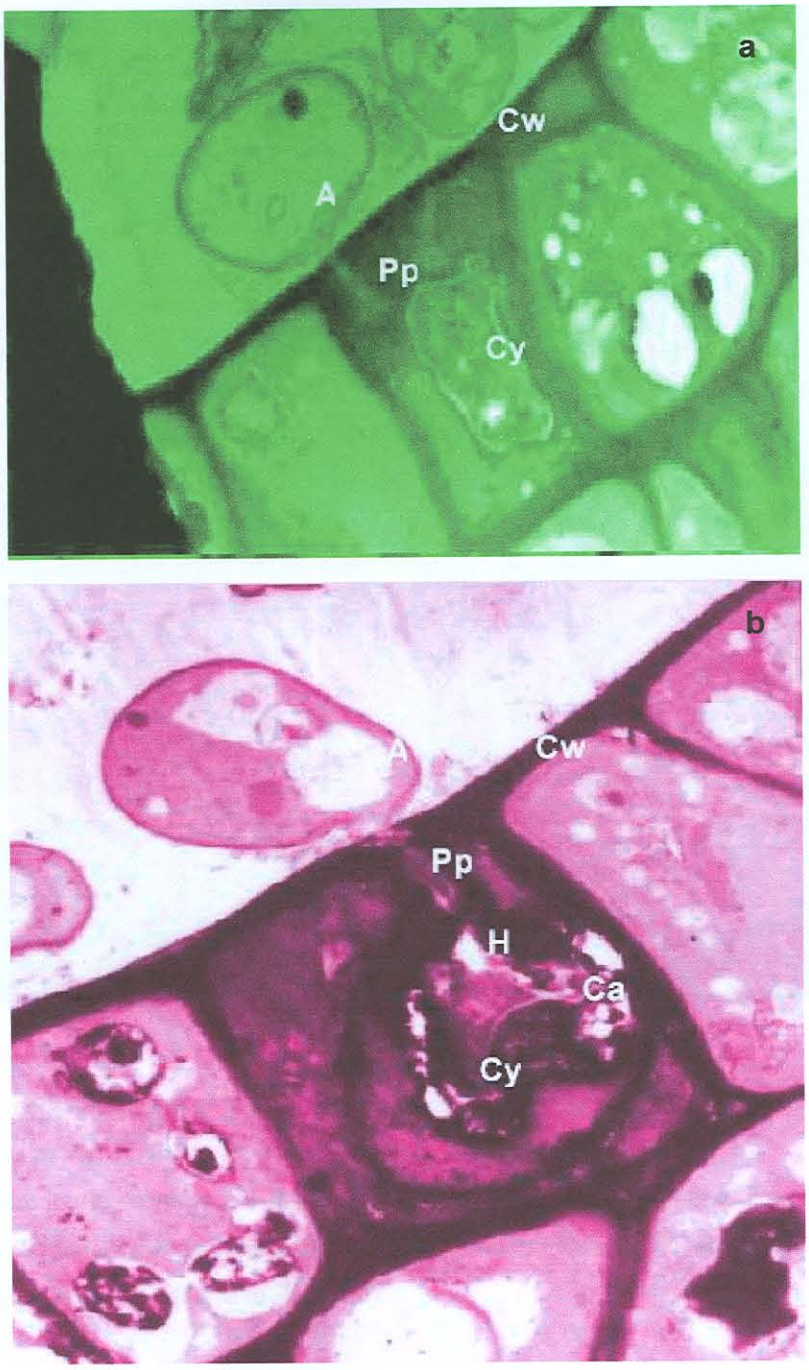


Figure 3.1 Scanning electron micrographs of germinating conidia of *Oidium anacardii* on young cashew leaves. a) Clusters of conidia on the upper leaf surface; b) Wax layer degradation by conidia; c) Elongation of primary germ tube; d) Elongated primary germ tube; e) Differentiation into an appressorium and f) Mature, expanded appressorium. The arrows indicate degraded area b) or fungal structures c) to f).



A = apressorium; Ca = cytoplasm aggregates; Cw = Thickened cell wall;
Cy = cytoplasm; Pp = penetration peg; H = haustorium

Figure 3.2 Transmission electron micrographs of *Oidium anacardii*, infection process 18 hpi, showing a) Penetration of haustorium into epidermal cells of clone Corane 2 and cytoplasm aggregation beneath haustorium. Mag. 3780x. b) Advanced infection with dense cytoplasm aggregation, clone H1. Mag. 4515x.

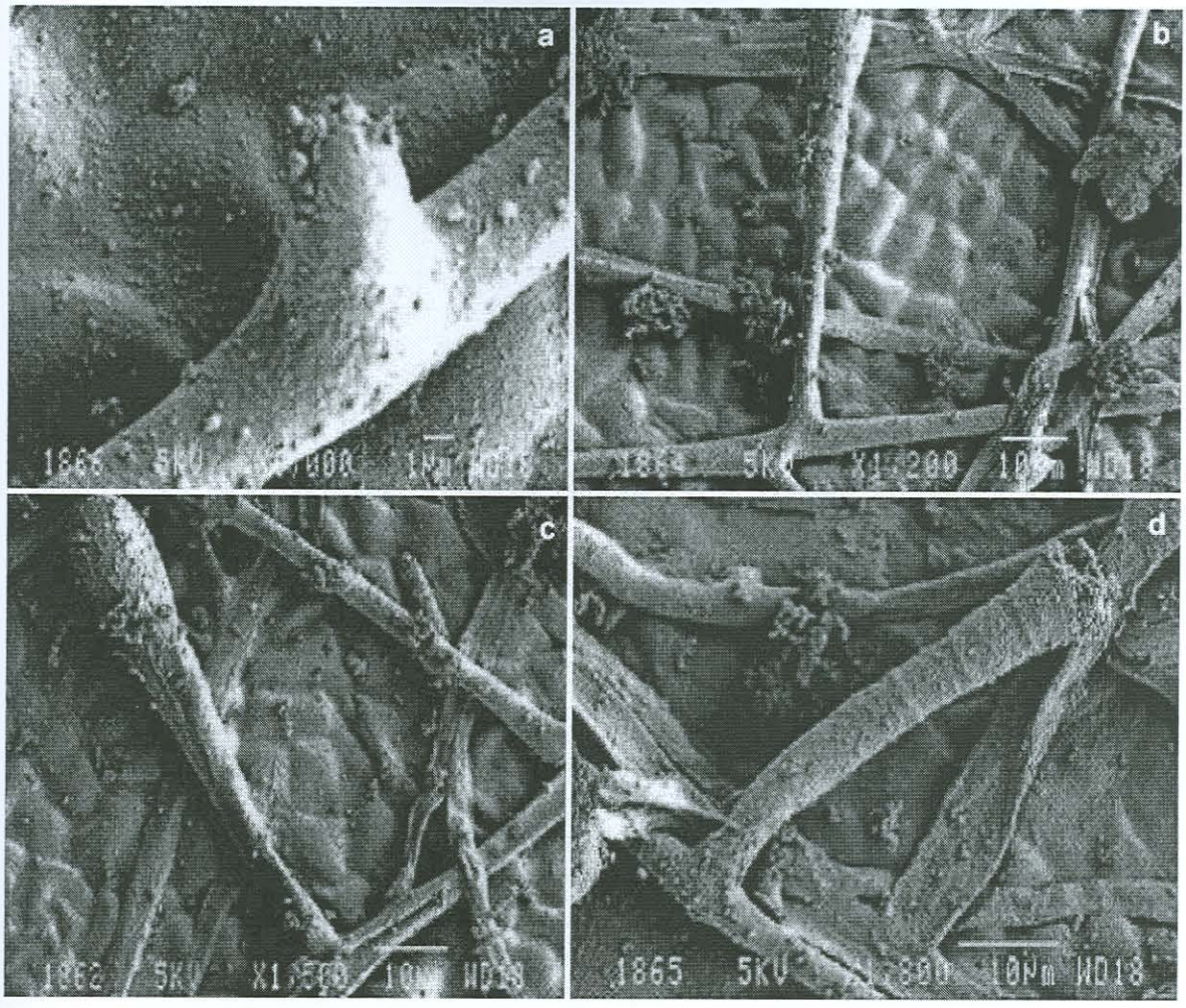


Figure 3.3 Scanning electron micrographs of *Oidium anacardii* condiophore and conidium development 72 hpi.on leaves of clone H1 a) Conidiophore initiation; b) Differentiating conidium with a straight basal cell; c) Conidium maturation; d) Conidiophore with conspicuous septa after conidium release. The arrows indicate fungal structures referred to in a) to d).

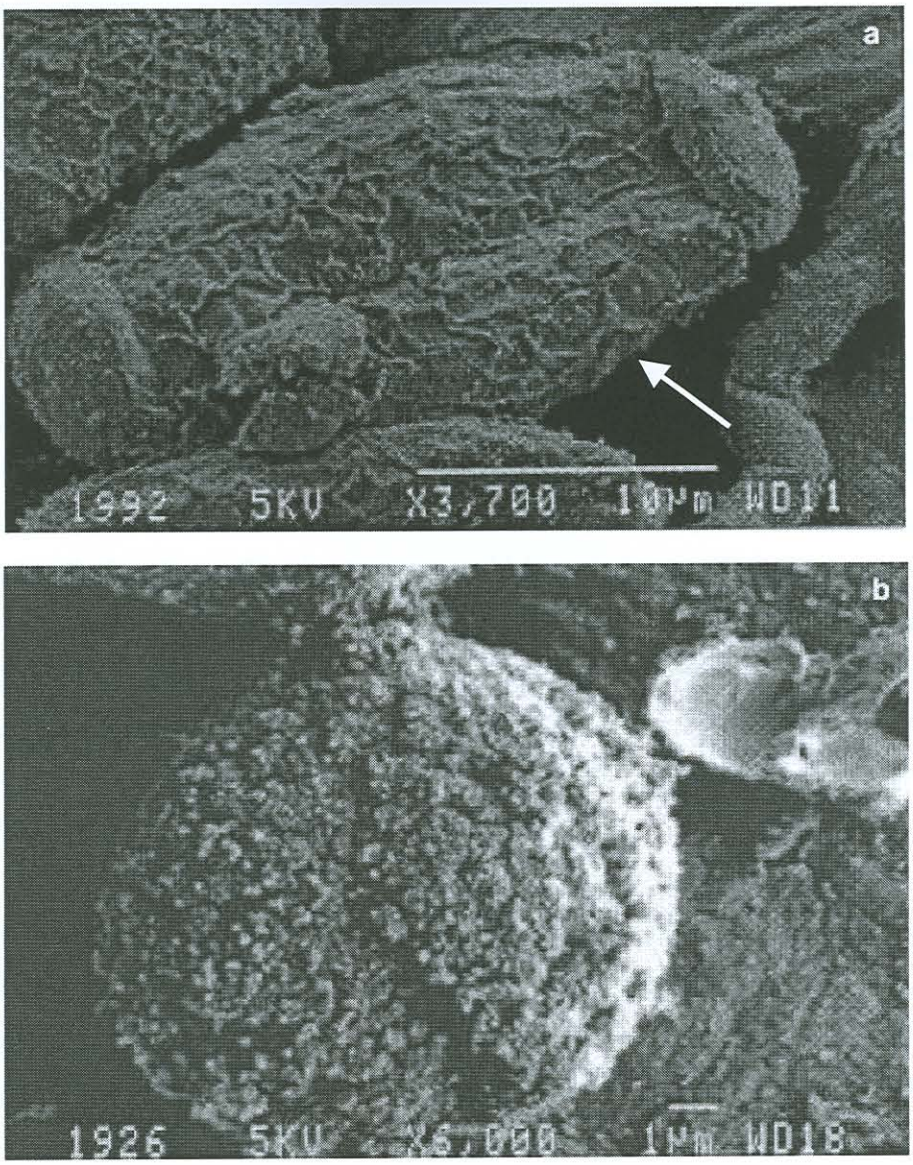


Figure 3.4 Scanning electron micrographs of *Oidium anacardii* conidial structures on leaves of clone H1 a) Outer wall wrinkles on collapsed conidium; b) Conidial septum. Arrows indicate the wrinkles a) and septum b).

Conidial structures

Conidia are hyaline and elliptical in shape. The outer wall on collapsed conidia consisted of a dense honey-combed wrinkling (Fig. 3.4a) and the pattern observed on a turgid conidial septum was characterised as smooth (Fig. 3.4b), i.e. neither verrucose nor particularly protuberant following Cook's SEM description of conidia.

DISCUSSION

The species *O. anacardii* was originally described by Noack in 1898 (Castellani & Casulii, 1981; Waller *et al.*, 1992; Shomari, 1996). Based on light microscopy, Castellani and Casulii (1981) provided a preliminary review on the morphology of this pathogen: The mycelium is superficial and abundant. The hyphae are whitish to light-grey in colour, septate with a diameter of about 3.5 µm. Conidiophores are short, measuring up to 45 µm in length with one or two basal cells. In this study, SEM and TEM were employed to further describe the fungus' morphology and its development on the leaf surface of three different cashew clones. Our observations on the external characteristics of the fungus were similar to those of previous researchers.

In the present study, conidia were found to have commenced germination 4 hpi, regardless of the clone. This confirms prior observations made *in vitro* on the same pathogen, where conidia germinated 4 hpi under environmental conditions of 15-35°C and 20 to 100% relative humidity (Shomari & Kennedy, 1998; Shomari & Kennedy, 1999). Similarly, Leinhos *et al.* (1997), working with another powdery mildew (*Uncinula necator* Schwein.) on grapevine leaves, found that the pathogen produced a germ tube within 4 hpi. Earlier germination 1-2 hpi has been reported for other powdery mildews (Plotnikova *et al.*, 1998).

To our knowledge, none of the earlier studies on *O. anacardii* has attempted to examine, at microscopic level, the relationship between the process of conidial germination and hosts of different resistance levels. Reported comparative studies on the relationship between different varieties and the pathogen have been based on superficial host tissue coverage by actively sporulating mycelia (Nathaniels, 1996; Sijaona *et al.*, 2001). In some cases, a reduced rate of germination is associated with the host's partial resistance to infection (Aist & Bushnell, 1991). None of the tested clones expressed any morphological or temporal differences upon conidial germination on the leaf surface which could be related to resistance

or tolerance to the disease. Therefore, our results suggest that germination of individual conidia on the host surface does not necessarily determine the severity of the disease. Disease severity is possibly determined once the pathogen has penetrated the leaf and organic interactions between the pathogen and the host are established.

Partial resistance to infection by powdery mildews can be determined by the failure to produce haustoria or having reduction in their size and development of papillae. More commonly, slower colony development without a hypersensitive reaction in the host, has been attributed to partial resistance (Aist & Bushnell, 1991). It has been hypothesized that tolerance to cashew powdery mildew is characterised by a slower development of infection and consequently slower growth of mycelium (Martin *et al.*, 1997). The hypothesis was supported by a higher level of cytoplasmic reaction by the tolerant clone H1 compared to the others. Possibly a more dense cytoplasm restricts the availability and absorption of nutrients through haustoria and consequently reduced development of the pathogen.

Our study illustrates the mechanism of infection. No obvious clonal differentiation could be made. However, more dense cytoplasm on clone H1 may be associated with a higher concentration of tannins (Coetzee, 2000, personal communication) which in turn may be involved in a biochemical response. Kanter *et al.* (1996) found that tannins conferred chemical and physical properties which protected *Vicia faba* L. germinating seeds against fungal infection. Spiers *et al.* (1998) also found association of these compounds with seasonal host tolerances of various plant species to fungal diseases. Finally, cashew varieties are known to possess different levels of tannins (Ferrão, 1995). This could also explain the resistance observed in mature tissues of all clones in the field. Nevertheless, the potential role of tannin production as defense strategy against *O. anacardii* infection requires further investigation.

Previous observations by Castellani and Casulii (1981) and Shomari (1996) revealed that *O. anacardii* possesses a short conidiophore with one or two basal cells. In our observations, the structure of the conidiophore coincided with the description given above, except that only one straight basal cell was observed. This type of conidiophore has also been reported for other powdery mildew fungi (Celio & Hausbeck, 1997). *Oidium anacardii* conidia are ellipsoidal, unicellular, hyaline, thin walled (Shomari, 1996) and produced in short chains (Waller *et al.*,

1992) of 4-8 spores (Ponte, 1984). Our findings support this description. Shomari (1996) further described that the conidia mature singly.

Under high humidity, two or three conidia may mature simultaneously on one conidiophore (Castellani & Casulii, 1981). The conidia produce a short germ-tube which terminates in a lobate appressorium measuring 8-10 µm (Shomari, 1996). Our observations agree with the above description. However, in our work the germ-tube appeared to be quite long. In addition, the SEM observations revealed for the first time that the conidial outer wall pattern is honeycomb ornamented with whorled or smooth septa according to the technique adopted in this study. The findings also confirm that separation of anamorphs with whorled septa from those with fibrillar septa is the most fundamental difference visible by SEM (Cook *et al*., 1997).

On the basis of their observations, Castellani and Casulii (1981) concluded that *O. anacardii* belongs to the *Pseudoidium* and fitted well with group A of the classification made by Zaracovitis (1965) cited by Shomari, 1996. Based on the pattern seen on turgid conidia on septa and on outer conidial walls, our investigation revealed that *O. anacardii* fits in well with the new classification proposed by Cook *et al*. (1997) as a member of *Oidium* subgenus Pseudoidium (Y.S. Paul & J.N. Kapoor) comb. Et. stat. Nov. (Holomorph *Erysiphe* Sect. *Erysiphe* U.Braun).

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CHAPTER 4

EPIDEMIOLOGICAL STUDIES OF CASHEW POWDERY MILDEW (*OIDIUM ANACARDII* NOACK) IN SOUTHERN MOZAMBIQUE

ABSTRACT

Regional and seasonal variations of climatic conditions are frequently reflected in differences of powdery mildew disease patterns over time. In this study, we evaluated the effect of climatic conditions on cashew powdery mildew (caused by *Oidium anacardii* Noack) progress on inflorescence tissue. The experiment was conducted at Ricatla Cashew Research Station (Maputo). Labelled shoots were assessed using a standard scale at seven day intervals from mid-July 1999 to August 2000 with interruption only during the non-flowering period. Meteorological data including air temperatures, relative humidity and dew points were collected from within the tree canopy using computer programmed sensors. Results showed that the powdery mildew epidemic progressed as a bi-modal epidemic; the first main epidemic built up from the end of June towards the end of September, while the second minor peak was reached in November. The onset of the epidemic did not start until the maximum average temperature was below 30°C and the average temperature equalled 20°C. Similarly, the prevailing average relative humidity had to be equal or above 80% and average dew point below 15.

INTRODUCTION

Cashew (*Anacardium occidentale* L.) originated and dispersed from north-east Brazil (Lopes *et al.*, 1993; Milheiro & Evaristo, 1994; Ferrão, 1995). During the sixteenth century, the Portuguese disseminated the species throughout Africa and Asia, i.e. Mozambique, Tanzania and India, while Spanish sailors introduced cashew to Panama and Central America (Ferrão, 1995; Behrens, 1996). Today, cashew is cultivated throughout the tropical world at low altitude and commonly near the Atlantic and Indian oceans (Milheiro & Evaristo, 1994; Ferrão, 1995). Besides the importance of its status as earner of valued foreign exchange,

cashew also provides employment, thereby stimulating the economy of developing countries particularly Asia, Africa and South and Central America (Rickson & Rickson, 1998). However, significant losses have been recorded in east Africa due to powdery mildew (*Oidium anacardii* Noack) (Castellani & Casulii, 1981; Milheiro & Evaristo, 1994; Nathaniels, 1996; Topper *et al.*, 2000).

In Mozambique, cashew powdery mildew remained negligible until the early 1970's (Milheiro & Evaristo, 1994), when its severity increased, causing a serious decline in production (Anon, 1999). It is suggested that climatic changes may have favoured dispersal and infection (Uaciquete, 1997). Inappropriate cultural practices motivated by difficulties in nut marketing, possible emergence of a new and virulent pathotype and the narrow genetic base of the present cashew populations are the major causes for the continuous recurrence of the disease (Milheiro & Evaristo, 1994; Prasad *et al.*, 2000).

Earlier studies (Shomari & Kennedy, 1998) demonstrated that *O. anacardii* conidial germination and cashew tissue infection by this pathogen occured over a wide range of temperature and humidity. Powdery mildew development is therefore influenced by environmental conditions that affect the viability of the pathogen (Shomari & Kennedy, 1999). In general, epidemic development is favoured by dry, cold conditions (Waller *et al.*, 1992; Milheiro & Evaristo, 1994).

Differences in weather conditions are common over time and between various cashew production areas (Nathaniels & Kennedy, 1996). Therefore, distinct patterns of epidemic development have been observed in different regions of Tanzania (Nathaniels *et al.*, 1993) and Mozambique (Nathaniels, 1994; Topper *et al.*, 2000). The above observations have led to the hypothesis that chemical applications for disease control would probably require more frequency in some regions than others as a consequence of climatic differences. In this context, the present study was aimed at understanding the progress of the disease on inflorescence tissue and its relationships with climatic parameters in the southern part of Mozambique.

MATERIALS AND METHODS

The experiment was conducted at Ricatla Cashew Research Station, 25 km north of Maputo, during the 1999/2000 and 2000/2001 crop seasons on 25-30 year old trees. The plantation was originally established by grafting for the purpose of germplasm preservation. Therefore, the trees selected represented various cloned land-types.

The methodology followed is essentially based on the work of Shomari and Kennedy (1999) but with an increased number of sampling points. Ten replicate plants were randomly selected within a block of 144 trees. Ten shoots per tree were labelled prior to the onset of growth. Thus, a total of 100 randomly distributed buds on both shady and sunny peripheral sides of the tree were targeted. Labelled shoots were assessed at seven-day intervals from mid July 1999 to August 2000 with interruption during the non-flowering periods.

At each assessment date, the severity of powdery mildew on panicles was evaluated using the cashew blossom scale of Nathaniels (1996). The scale assesses the percentage of diseased blossom components (florets and buds) out of the total number of florets and buds. It consists of a visual severity rating of 0-6; where 0 = no active disease, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-99% and 6 = 100% diseased blossom (Appendix.1). The overall mildew score for a given observation date on a tree was calculated using the following procedure of Masawe *et al.* (1997): The median of the disease severity scale was: 0 (for 0%); 5.5 (for 1-10%); 18 (11-25%); 38 (26-50%); 63 (51-75%); 87 (76-99%) and 100 (100%). The frequency of panicles scored was tabulated showing the date of observation and scores on a 0-6 scale. The formula multiplied each median by the frequency of panicles scored in that scale, summed and divided by the total sample value (Masawe *et al.*, 1997).

Meteorological data included air temperature, relative humidity and dew point and were collected using sensor devices (model HOBO H8, RH/Temp.) obtained from Onset Computer Corporation, Massachusetts, USA. The sensors were placed at a height of 6 m within the tree canopy. They were protected from direct sunlight and rain through special metallic funnels (Michael *et al.*, 1996). The devices were computer programmed to record data at 15 min intervals in order to assess changes in the above parameters.

RESULTS

Detailed meteorological data including standard deviations are presented in Table 4.1. The pattern of disease severity during the late 1999/2000 season is given in Fig. 4.1a. The post-terminal stage of the epidemic in 1999 indicates that a decline in severity was observed from week 44 to the end of the flowering season (November, week 48). No data was recorded between week 48/1999 (end of flowering season) and week 23/2000 (beginning of new flowering season). The actual epidemic (Fig. 4.1b) progressed rapidly from June (week 25/2000) to August (week 30/2000), when 100% inflorescence florets and buds had been infected.

No direct relationship between powdery mildew severity and dew point could be made. However, weekly dew point equal to or below 15 appeared to be associated with the onset or high levels of disease severity, weeks 1 to 12 and 42 to 52 (Fig. 4.2a).

Weekly mean maximum temperatures above 30°C was associated with absence or decrease in disease severity in weeks 13 to 42 (Fig. 4.2b). The onset of the measured disease epidemic was not observed until the average temperature was around 20°C, at week 42 (Fig. 4.2b). There was no evidence of a direct relationship between any of the above climatic parameters and disease severity over the year.

Weekly average relative humidity decreased to levels below 80%. The end of the rainy season was associated with disease outbreaks or high severity levels during weeks 1 to 12 and 42 to 52 (Fig. 4.2b). No evidence of direct relationship between the relative humidity and disease severity could be detected.

DISCUSSION

The present data show that for the study period, the powdery mildew epidemic progressed in the southern part of Mozambique as a typical bi-modal epidemic. Towards the end of the crop season, i.e., November, 1999, a sharp severity decline was observed due to lack green florets on the panicle. Therefore when new flowers emerged from the same panicle under observation, then powdery mildew pathogen activity restarted. This abnormality is probably a

response of the tree to the severe attack of the pathogen on the first set of florets. The first main epidemic built up from the end of June towards the end of September. The second minor peak was reached in November, after which it declined until the following season. Our findings are similar with previous studies (Nathaniels *et al.*, 1993) at Naliendele, Tanzania, where the disease appeared for the first time on flowers around the first week of July. The intensity reached almost 100% by mid-August. These authors also found that terminal flower disease was lower (75-80%) than during the first flowering flush. It is noted from our findings that symptoms of powdery mildew occur on panicles up to November. This period coincides with that registered for the emergence of panicles in the southern part of Mozambique (Fig. 2.1 Chapter 2) (Milheiro & Evaristo, 1994). Therefore, the results suggest that late decline of the disease is related to limited availability of susceptible tissue. Higher prevalence of the disease in the south is probably related to a longer period of availability of susceptible tissues.

Our results further showed that the onset of cashew powdery mildew epidemic does not start until conditions of a mean maximum temperature below 30°C, prevailing mean maximum relative humidity above 80% and mean maximum dew point below 15 are reached. Castellani and Casulii (1981) reported conidial germination at a RH ranging between 88 and 100% with an optimum around 95%. Nathaniels *et al.* (1993) stated that exposure to low humidity may rapidly reduce viability through shrivelling and loss of conidial turgidity. However, cashew powdery mildew conidia were demonstrated to germinate under humidity conditions from 20 to 100% (Shomari & Kennedy, 1998). Therefore it is likely that in our observations, disease epidemic onset may have been trigged by the levels of maximum temperature combined with maximum dew point rather than by the changes in levels of minimum and maximum relative humidity. During our study, the average relative humidity prevailed between 60 and 80% throughout the year. This encompasses what is reported for Mozambique (73 to 79%) (Milheiro & Evaristo, 1994). The wide interval observed may be explained by the occurrence of abnormally high rainfall (floods) during the study period.

Previous observations on inoculated shoots showed that at 15 or 35°C, mildew development does not occur (Shomari & Kennedy, 1998). In addition, a decline in the rate of conidial germination below 20°C and above 35°C or no germination at all below 10 or above 40°C, has been reported (Nathaniels *et al.*, 1993). Furthermore, Gupta (1988) studying mango powdery mildew (*Oidium mangiferae* Berth.) reported no infection at 10 and 35°C, but that infection

could take place even at relative humidities as low as 15%. Therefore, it can be concluded that maximum and minimum temperatures are the key parameters that restrict powdery mildew epidemic development. Similar observations were made by Xu and Butt (1998), who studied the effect of temperature and atmospheric moisture on early growth of apple powdery mildew caused by *Podosphaera leucotricha* Ell. & Everh.

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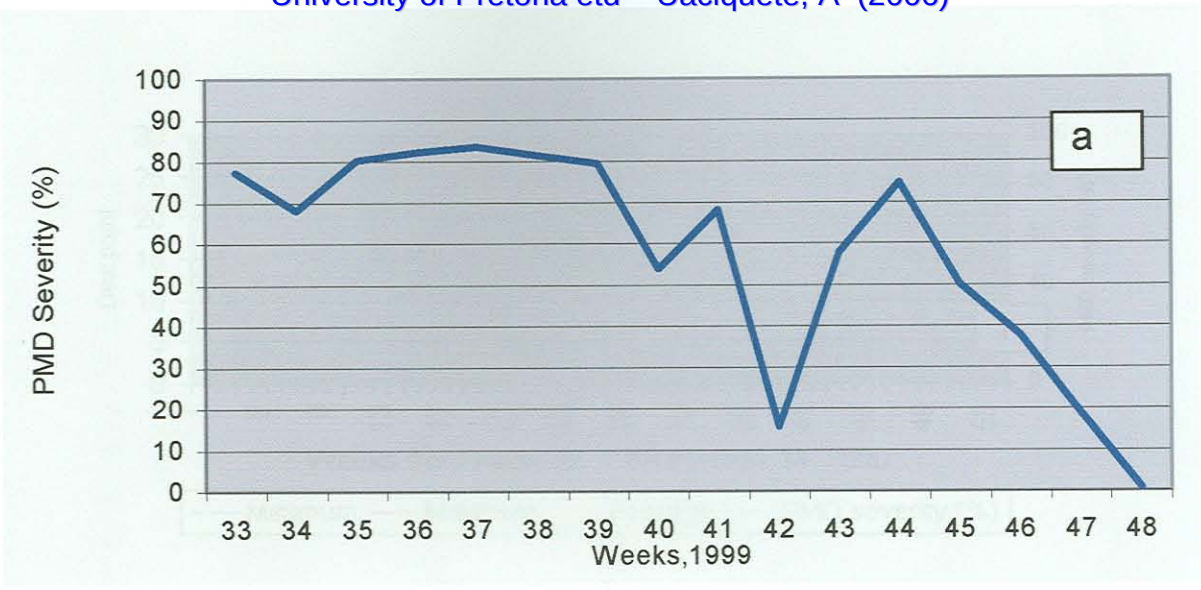

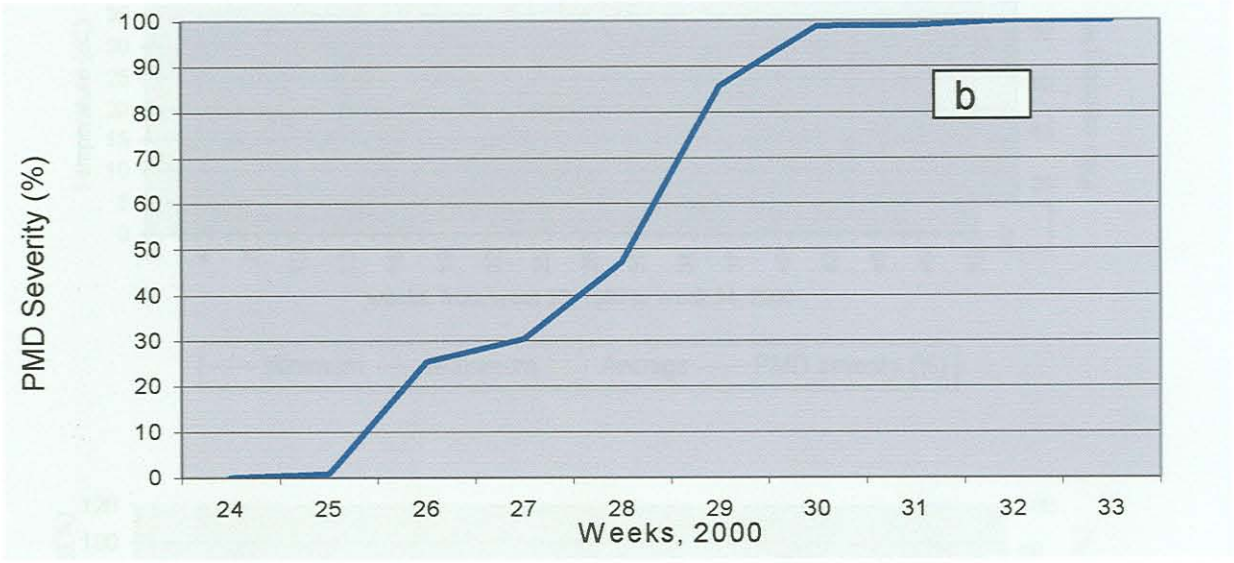

Figure 4.1 Progress of powdery mildew disease (PMD) development on cashew (*Anacardium occidentale* L.) flowers at Ricatla research station from a) August to November, 1999 and b) from June to August 2000, over two consecutive crop seasons.

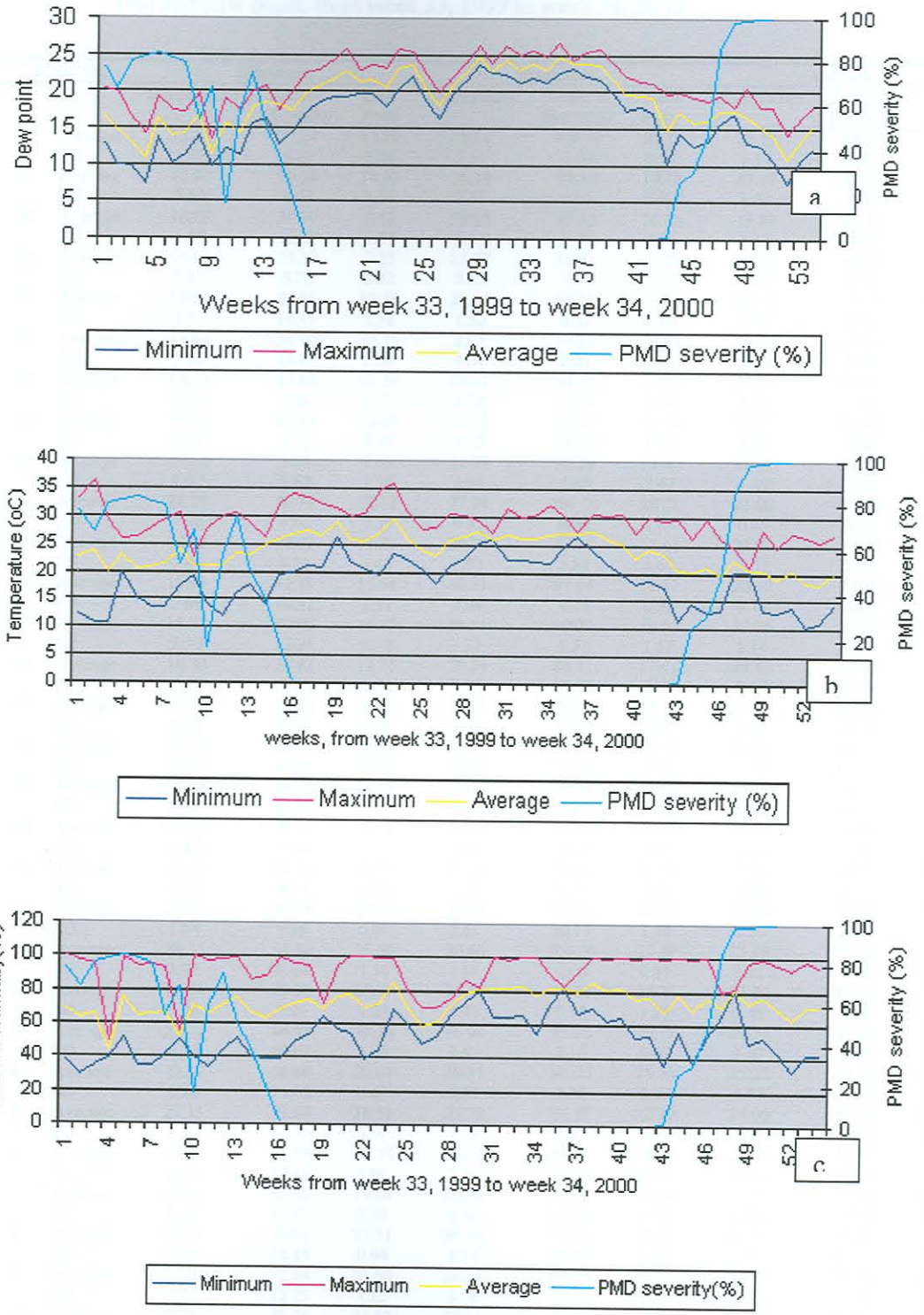

Figure 4.2 Progress of powdery mildew disease (PMD) development on cashew flowers at Ricatla Research station during the 1999/2000 and 2000/2001 crop seasons, in relation to various climatic parameters at mean minimum, average and maximum levels: (a) Dew point; (b) Temperature and (c) Relative humidity.

Table 4.1 Weekly average and standard deviation (SD) for temperature (°C), relative humidity (%) and dew point, from week 33, 1999 to week 34, 2000

Year	Week No.		Minimum			Maximum			Average		
1999			Temperature	Relative Humidity	Dew Point	Temperature	Relative Humidity	Dew Point	Temperature	Relative Humidity	Dew Point
	33	Average	12.37	37.74	12.70	33.21	100.00	20.20	22.79	68.87	16.45
		SD	1.27	6.23	1.08	2.51	0.00	0.95	1.17	3.12	0.89
	34	Average	10.80	29.24	10.02	36.14	97.49	19.75	23.47	63.36	14.89
		SD	3.42	4.42	1.29	1.11	6.65	0.90	1.50	4.00	0.58
	35	Average	10.59	34.96	9.61	29.08	95.33	16.55	19.84	65.14	13.08
		SD	1.85	10.19	1.58	6.14	11.80	2.90	3.12	8.87	1.30
	36	Average	19.92	39.39	7.25	25.87	48.81	14.22	22.89	44.10	10.73
		SD	1.88	5.70	3.72	3.15	7.94	4.15	1.78	6.12	3.16
	37	Average	15.01	50.91	13.58	26.24	99.76	19.22	20.63	75.29	16.40
		SD	1.75	10.41	2.71	1.50	0.52	0.98	1.49	5.32	1.71
	38	Average	13.62	34.90	10.23	27.7	94.33	17.53	20.75	64.61	13.88
		SD	3.10	10.88	3.39	4.04	7.21	1.67	3.29	7.74	2.42
	39	Average	13.42	35.43	11.24	29.31	94.63	17.25	21.37	65.03	14.25
		SD	2.01	9.89	1.16	5.72	6.06	2.14	3.62	6.66	1.37
	40	Average	17.41	41.66	13.98	30.53	92.19	19.60	23.97	66.92	16.79
		SD	4.37	9.75	2.39	3.02	11.21	1.70	3.24	6.08	1.96
	41	Average	19.21	50.3	9.69	22.59	55.29	13.40	21.02	52.14	11.47
		SD	1.60	3.62	2.28	1.66	4.40	2.47	1.49	3.62	2.18
	42	Average	14.25	40.74	12.13	27.74	100.00	19.02	21.00	70.37	15.58
		SD	1.06	4.60	2.39	0.93	0.00	1.04	0.75	2.13	1.33
	43	Average	12.07	34.07	11.33	29.96	97.60	17.43	21.02	65.84	14.38
		SD	3.84	6.76	1.48	3.97	4.88	1.67	2.57	2.38	1.41
	44	Average	15.93	43.79	15.64	30.51	97.64	19.87	23.22	70.71	17.75
		SD	2.60	10.92	2.31	2.64	4.28	0.97	0.73	4.87	1.45
	45	Average	17.57	50.99	16.42	28.54	99.70	20.83	23.06	75.34	18.63
		SD	2.99	13.21	1.77	1.37	0.51	1.53	1.14	6.09	1.85
	46	Average	14.30	39.82	12.76	26.24	86.11	17.41	24.86	66.73	18.12
		SD	5.19	9.95	1.30	4.54	21.56	2.07	2.14	8.79	1.08
	47	Average	19.54	39.23	14.63	31.98	88.06	20.17	25.76	63.64	17.40
		SD	2.99	9.73	3.22	5.51	19.09	3.86	3.33	13.09	3.06
	48	Average	19.70	39.07	16.90	33.91	99.31	22.58	26.80	69.19	19.74
		SD	2.21	7.64	2.13	2.00	1.32	1.19	1.72	3.46	1.57
	49	Average	21.06	48.71	18.61	33.53	95.80	23.02	27.30	72.26	20.81
		SD	0.88	15.23	2.41	4.50	5.20	1.41	2.52	9.37	1.74
	50	Average	20.68	53.73	19.11	32.00	95.29	24.23	26.34	74.51	21.67
		SD	2.47	11.04	1.56	3.63	11.18	1.17	2.54	7.06	1.01
	51	Average	26.07	64.16	19.09	31.42	72.33	25.78	28.83	69.22	22.74
		SD	1.15	6.17	2.50	1.43	3.39	1.54	0.80	4.25	1.41
	52	Average	21.82	56.89	19.61	29.93	95.10	23.03	25.88	75.99	21.32
		SD	1.64	6.36	0.92	2.51	10.17	1.75	1.30	3.41	1.24
2000	1	Average	20.35	54.80	19.66	30.60	100.00	23.59	25.48	77.40	21.62
		SD	1.27	6. 64	1.56	1.01	0.00	0.81	0.91	3.32	1.07
	2	Average	19.64	39.21	17.77	34.75	99.86	23.25	27.20	69.54	20.51
		SD	1.96	7.96	1.24	3.53	0.35	1.21	2.49	4.05	1.04
	3	Average	23.19	44.91	20.36	35.94	98.86	25.77	29.57	71.89	23.06
		SD	0.72	10.67	1.39	2.82	2.10	0.69	1.57	5.00	0.68
	4	Average	22.37	68.46	22.07	30.87	99.00	25.30	26.28	84.72	23.58
		SD	0.76	11.03	0.67	3.00	2.14	0.60	1.77	5.54	0.61
	5	Average	20.35	59.60	18.51	27.77	79.77	22.20	24.06	69.69	20.36
		SD	1.41	12.30	1.97	2.58	6.88	2.23	1.81	8.75	1.98
	6	Average	18.01	47.53	16.30	28.11	69.60	19375	23.06	58.56	18.03
		SD	0.70	12.43	1.96	2.79	12.33	1.07	1.51	11.55	1.25
	7	Average	20.95	52.30	19.68	30.50	69.37	22.04	25.73	60.84	20.86
		SD	2.15	11.84	2.49	2.30	15.89	2.33	1.49	13.35	2.38
	8	Average	22.64	66.0	21.31	30.18	75.60	23.86	26.41	70.80	22.58
		SD	0.28	18.85	0.96	3.17	15.70	1.06	1.56	17.23	0.95
	9	Average	25.29	72.64	23.50	29.27	86.59	26.14	27.24	78.33	24.74
		SD	1.80	12.25	1.12	3.50	8.93	2.06	2.00	8.60	0.66
	10	Average	25.91	79.36	22.52	27.14	82.33	23.87	25.96	80.58	22.64
		SD	1.91	3.79	2.12	1.97	3.72	2.38	1.91	3.64	2.20
	11	Average	22.26	64.71	22.26	31.58	100.00	26.35	26.92	82.36	24.31
		SD	1.10	8.58	1.27	3.34	0.00	1.49	2.14	4.29	1.36
	12	Average	22.37	64.11	21.14	29.98	98.70	24.90	26.17	81.41	23.02
		SD	1.25	7.60	1.96	1.81	2.83	1.76	1.41	4.85	1.81
	13	Average	22.20	65.94	21.90	30.26	100.00	25.64	26.23	82.97	23.77
		SD	0.81	6.98	0.64	1.06	0.00	0.75	0.67	3.49	0.63

14	Average	21.77	55.01	21.27	31.99	100.00	24.66	26.88	77.51	22.96
	SD	0.56	4.04	0.60	0.74	0.00	0.61	0.47	2.02	0.50
15	Average	24.59	72.16	22.54	30.10	90.51	26.61	27.19	82.09	24.69
	SD	3.00	8.30	0.79	2.53	10.98	2.00	2.17	5.87	1.02
16	Average	26.45	81.43	23.20	27.46	82.81	24.28	27.03	81.90	23.76
	SD	0.75	2.00	0.46	0.85	1.80	0.46	0.78	1.88	0.44
17	Average	23.91	66.57	22.12	30.67	91.79	25.45	27.29	79.18	23.79
	SD	1.77	12.86	0.68	3.91	9.50	2.50	1.25	3.56	1.34
18	Average	21.88	69.83	21.55	30.28	100.00	25.80	26.08	84.91	23.68
	SD	0.91	10.53	1.24	2.59	0.00	1.08	1.57	5.26	0.81
19	Average	19.86	61.96	19.53	30.53	99.47	23.90	25.19	80.71	21.72
	SD	0.83	14.54	0.73	3.33	1.29	0.95	1.68	7.36	0.55
20	Average	18.01	64.10	17.45	27.20	100.00	22.01	22.61	82.05	19.73
	SD	1.57	11.72	2.30	5.24	0.00	2.63	3.26	5.86	2.14
21	Average	18.72	52.61	17.94	29.97	99.69	21.42	24.23	75.63	19.39
	SD	1.54	4.93	1.65	1.61	0.50	1.86	1.37	2.45	1.70
22	Average	17.36	53.06	17.27	29.28	100.00	21.19	23.25	76.51	19.14
	SD	1.22	2.63	0.99	1.00	0.00	0.71	0.83	1.31	0.62
23	Average	11.42	36.16	10.16	29.74	99.11	19.55	20.58	67.64	14.85
	SD	2.13	9.99	1.67	3.53	2.17	5.12	1.57	5.12	2.74
24	Average	14.36	55.69	14.44	26.25	99.83	19.83	20.30	77.76	17.14
	SD	1.02	10.26	0.92	2.37	0.42	1.20	0.91	5.01	0.58
25	Average	13.04	38.21	12.55	29.46	98.93	19.25	21.25	68.57	15.90
	SD	1.83	10.49	1.42	1.64	2.14	1.87	1.36	5.33	1.46
26	Average	13.26	52.94	13.33	26.08	99.83	18.85	19.67	76.39	16.09
	SD	1.74	12.90	1.37	2.41	0.42	0.47	1.24	6.40	0.48
27	Average	20.02	65.70	15.72	24.45	79.41	19.43	22.35	71.34	17.47
	SD	3.22	14.88	2.08	3.95	9.16	2.84	2.12	8.41	0.99
28	Average	20.14	80.24	16.98	21.00	81.31	17.95	20.57	80.78	17.47
	SD	0.13	0.55	0.21	0.13	0.51	0.21	0.10	0.45	0.16
29	Average	13.07	48.77	12.93	27.57	97.44	20.54	20.32	73.11	16.74
	SD	3.73	14.51	2.48	4.86	6.26	5.49	1.77	4.93	2.50
30	Average	12.98	52.10	12.63	24.68	99.66	17.93	18.83	75.88	15.28
	SD	1.54	8.82	0.81	0.57	0.54	0.87	0.88	4.46	0.51
31	Average	13.81	43.71	10.50	26.98	93.51	17.93	20.40	70.11	14.21
	SD	3.97	15.34	6.95	2.65	7.46	3.79	2.95	10.24	5.02
32	Average	10.31	32.77	7.42	26.37	92.80	14.15	18.34	62.79	10.78
	SD	1.74	12.69	2.46	2.98	9.39	3.39	1.91	9.65	2.48
33	Average	10.98	43.01	10.75	25.46	98.01	16.56	18.05	70.99	13.38
	SD	1.94	8.00	1.01	1.92	3.51	1.58	1.60	4.63	0.99
34	Average	14.46	43.09	12.40	26.74	94.31	18.16	20.12	70.83	15.38
	SD	3.36	10.13	2.10	1.44	13.44	1.58	1.54	7.06	1.39

CHAPTER 5

IN VITRO AND *IN VIVO* SCREENING OF NATURAL AND COMMERCIAL ANTAGONISTS AGAINST THE CASHEW POWDERY MILDEW PATHOGEN, *OIDIUM ANACARDII* NOACK

ABSTRACT

Current integrated cashew management strategies for powdery mildew (*Oidium anacardii* Noack) control are based on sanitation, use of tolerant varieties and most importantly recurrent application of fungicides such as sulphur, Anvil and Bayfidan. Global awareness on the negative impact of agrochemicals on the environment and human health as well as build-up of pathogen resistance, associated with limited effectiveness of some fungicides and reluctance of the chemical industry in developing new products, have led to the development of new alternatives for disease control. Biological control is one such option. The aim of this study was therefore to screen *in vitro* and *in vivo*, newly isolated and commercial potential biocontrol agents against cashew powdery mildew. From January to July 2000, natural potential antagonists were isolated from cashew leaves and florets. A total of 72 isolates were obtained and screened *in vivo* alongside three commercial biocontrol agents (*Bacillus subtilis*, *B. licheniformis* and *Candida saitoana*) against *O. anacardii*. Leaf disc and panicle assay techniques were used and a recommended fungicide Bayfidan (triadimenol 25% EC) was used as a positive control. The investigation illustrated that none of the potential antagonists isolated from cashew leaves and florets had significant inhibitory effects on *O. anacardii* spore germination and hyphal growth. All commercial antagonists significantly reduced the length of the germ-tube within 24 hours post inoculation. The biocontrol agent *B. licheniformis* also significantly reduced the rate of branching of the primary hyphae and was thus established as the most promising antagonist for field trials.

INTRODUCTION

Several reasons for the declining production of cashew in eastern Africa have been proposed (Uaciquete, 1997; Mniu, 1998; Shomari, 1998). Of these, powdery mildew disease, caused by the fungus *Oidium anacardii* Noack, was regarded as the most important (Castellani & Casulii, 1981; Intini & Sijaona, 1983; Waller *et al.*, 1992; Shomari, 1996). Losses of between 50 and 70% have been attributed to the disease (Milheiro & Evaristo, 1994), which has become endemic to the region (Nathaniels, 1996). Powdery mildew disease management relies primarily on the application of sulphur dust or new systemic fungicides such as triadimenol EC 250 g a.i./l (Bayfidan), hexaconazole EC 50 g a.i./l (Anvil) and pencanozole EC 100 g a.i./l (Topas) (Waller *et al.*, 1992; Martin *et al.*, 1997; Smith *et al.*, 1997).

Global awareness of the adverse effect of synthetic chemical residues on human health and the environment (Waller *et al.*, 1992; Smith *et al.*, 1997), build-up of resistance by the pathogen (Korsten *et al.*, 1995; Dik *et al.*, 1998), limited control of fungicides and reluctance of chemical industries to invest in development of new products (De Jager, 1999) have necessitated a search for alternative non-chemical methods (Korsten *et al.*, 1995; Dik *et al.*, 1998; De Jager, 1999).

Biocontrol of fruit and leaf diseases through the use of antagonistic microorganisms has recently emerged as a viable disease management strategy (Korsten *et al.*, 1995). In this context, biological control of various biotrophic plant pathogens has been extensively investigated and reviewed (Pusey & Wilson, 1984; Sundheim, 1986; Korsten *et al.*, 1991; Elad *et al.*, 1996; Kiss, 1997; Dik *et al.*, 1998; Koumaki *et al.*, 2000). On mango (*Mangifera indica* L.), screening of bacteria isolated from the phylloplane for antagonism against bacterial black spot (*Xanthomonas campestris pv. mangiferaeindicae* (Patel, Moniz & Kulkarni) Robbs, Ribeiro & Kimura) resulted in identification of two isolates of *Bacillus licheniformis* (Weigmann) Chester (B250 and B251), which in the greenhouse completely inhibited the pathogen (Korsten *et al.*, 1992). In addition, the antagonists reduced powdery mildew (*Oidium mangiferae* Berth.) and anthracnose (*Colletotrichum gloeosporioides* Penz.) in preharvest integrated treatments (De Jager, 1999). Recently, in semi-commercial glasshouse trials, Dik *et al.* (1998) concluded that the yeast-like fungus *Sporothrix flocculosa* Traq. had potential for efficient biocontrol of cucumber powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht.:Fr.) Palacci. Unfortunately, to our knowledge, there is not

much information regarding mycoparasitism or other similar mechanisms of biological control for cashew powdery mildew. The only publication found thus far is the work of Casulii (1979), who speculated that the frequent presence of the hyperparasitic fungus *Cicinnobolus cesatii* De Bary, in association with powdery mildew, could in future constitute a basis for biological control of the disease. The aim of this study was therefore to screen *in vivo* natural and commercial potential antagonists against the cashew powdery mildew pathogen.

MATERIALS AND METHODS

Acquisition and preservation of antagonists: Epiphytic microorganisms were isolated from cashew leaves and florets (when available) of five randomly selected trees at the National Agronomic Institute, Mozambique. Samples were collected once each month, from January until July 2000. Ten powdery mildew infected leaves of approximately the same size and ten florets were picked at five points at eye level representing north, south, east and west and within the tree canopy. Thus, two sample units of each type (leaf or floret) were collected from each side of the tree. Leaves were handled by the petiole and florets by the pedicel, placed into sterile paper bags, transported to the laboratory in a cooler box and processed on the same day (Korsten *et al.*, 1995). Following the methodology described by Koomen and Jeffries (1993) entire leaves were manually shaken in 10 ml sterile distilled water for approximately 10 min. About 10 florets were directly vortexed in 10 ml sterile distilled water, followed by a dilution series. Sub-samples were spread onto either nutrient agar (NA, Oxoid) for isolation of bacteria or malt extract agar (MEA, Oxoid) with penicillin (30 mg/l) and streptomycin (50 mg/l) for isolation of yeasts and filamentous fungi. Bacterial single colonies were subcultured for purification and preservation. Fungal cultures were isolated by aseptically removing 0.5 cm^2 plugs from the edge of the actively growing colonies. All isolates were numerically coded, purified and stored at 4-5°C on respective growth media. Commercial biocontrol agents include *Bacillus licheniformis* previously isolated from mango leaves (lot 16, 27/7/2000, Korsten, L., Department of Microbiology and Plant Pathology, University of Pretoria), *Candida saitoana* Nakase & M. Suzuki from citrus fruit (lot CS-T2, 01/2000, Anchor Yeast, S.A), and *B. subtilis* (Ehrenberg) Cohn from avocado leaves (lot 3, Avogreen, 5/6/2000, Stimuplant CC, S.A.).

The host and inoculation of antagonists: Two approaches were followed to screen the effect of antagonists on powdery mildew disease development, namely the panicle and leaf disc assays. In the panicle assay, powdery mildew free panicles were cut just beneath the third leaf and transported in a cooler box to the laboratory where they were immediately placed in test tubes filled with sterile sorbitol (2% w/v). The cut section of each individual panicle was submerged to at least 10 cm deep. All test tubes were placed vertically in a wet sand-bed in the greenhouse. The sand-bed was watered to runoff at 9h00 and 16h00 to maintain approximately 90% relative humidity at temperatures of 25-28°C. Temperature and relative humidity in the greenhouse were monitored by a thermohygrometer (Type 252 44T 7d, Wilh. Lambrecht Gmbh, Goffingen). A completely randomised block design (Gomez & Gomez, 1984) was adopted. For each of the eigth experimental sets, 10 treatments were used in three replicates. Individual replicates consisted of three panicles in different test tubes. Treatments consisted of testing nine isolates separately as potential antagonists. A water treatment was included as a negative control. A total of 72 cashew isolates were tested as potential antagonists against cashew powdery mildew using the panicle assay technique.

Natural isolates were grown on NA or MEA media for 2 d (bacteria) and 7 d (fungi) respectively at 25-28°C before being scraped off the surface and suspended in sterile distilled water for immediate use. A day before inoculation with the pathogen, panicles were sprayed to runoff with either bacterial cells or fungal spores calibrated to concentrations of 10^7 cells and 10^6 spores or colony forming units per ml respectively (Koomen & Jeffries, 1993). The commercial antagonist *C. saitoana* was provided in granular formulation, thus 20 g of the granules were rehydrated in 316 ml of water, activated at 35°C for 20 min with no stirring and then poured into 9 l of water and stirred. A hand sprayer (500 ml) was used to apply either natural or commercial antagonists at recommended rates to each panicle.

The disc assay technique was adopted when disease free panicles became scarce in the field (November-April) and temperatures rose to above 40°C in the greenhouse. Under these circumstances, panicles could not be maintained surviving in sorbitol filled test tubes. Thus, reddish leaves from the second or third nodes of young healthy looking shoots were collected and superficially sterilised by immersion in 70% ethanol for 1 min (Pruvost & Luisetti, 1991). Leaf discs were excised with a 20 mm diameter cork borer which had been surface sterilised in a 0.5% sodium hypochlorite solution for 1 min, rinsed in sterile distilled water and air dried in the laminar flow (Celio & Hausbeck, 1997). The discs were placed on sterile Whatman

no.1 filter paper saturated with sorbitol (aqueous concentration 2% w/v) (Nathaniels *et al.*, 1993). A completely randomised block design with six treatments in three replicates was used. Each replicate consisted of three Petri dishes with three leaf discs in each. The treatments consisted of triadimenol 25% EC (Bayfidan) and bromuconozole 20 EC (Granit) at final concentrations of 15 ml/l and 0.15 ml/l respectively as positive controls, three commercial antagonists *B. licheniformis, C. saitoana* and *B. subtilis* and a negative water control. Commercial antagonists were prepared as described for the panicle assay technique and sprayed onto pathogen inoculated discs. The pathogen was inoculated on the 24 h pre-treated discs by releasing a single pipette drop of the inoculum on the disc surface.

Pathogen inoculation: Cashew foliage / panicles with natural mildew infection were selected 24 h before each inoculation date and shaken to dislodge old spores and encourage production of fresh spores overnight. Using the camelhair brush method (Shomari, 1996), inoculum was transferred from the source surface into 10 ml of sterile distilled water in order to standardise concentration. Thus, the concentration of the conidia was adjusted to 10^6 spores/ml using a haemacytometer. A single 5 ml pipette drop was released onto the surface of each disc. The plates were then positioned at an angle to allow a smooth absorption of excessive water by the filter paper beneath. All inoculated plates were incubated at room temperature with daylight for 24 h (Shomari & Kennedy, 1999). In the test tube trials, panicles were sprayed with a suspension of individual potential antagonists until runoff.

Observations and data analysis: Disease development on panicles was monitored daily and severity scores taken 10 d after pathogen inoculation when the untreated control reached maximum disease severity levels. In order to assess levels of the disease on individual panicles (panicle assay), a zero to six cashew blossom disease severity scale (Nathaniels, 1996; Masawe *et al.*, 1997) was used. Since the blossom disease severity scale could not be used for the leaf disc method, spores were removed 24 h after pathogen inoculation, using the cellotape technique (Nathaniels *et al.*, 1993). Cellotape was firmly pressed onto the inoculated side, then removed and mounted on a dry microscope slide. Three light microscope fields corresponding to one leaf disc were examined and at least 50 conidia per field were observed. A total of nine fields per treatment, per replicate were surveyed. From each slide, the following growth parameters at 200x magnification were noted: Total conidia observed, total germinated conidia, total conidia with two or more hyphae and the length (µm) of primary hyphae (Nathaniels *et al.*, 1993). To assist in continuous counting of spores, a

counting tool (Ferrari Statitest, Berlin, Germany) was used. Conidia germination was considered to have occurred when a new developing hypha, could be seen emerging from the spore (Isaac, 1998) with its length equal to at least half the width of the conidium (Celio & Hausbeck, 1997).

All data were transformed into percentages except for the length of the germ-tube. To transform data into percentages, the following equation was used: Total number of germinated conidia or total number of conidia with two or more hyphae, divided by total number of conidia observed times hundred. The percentages of powdery mildew on panicles were derived from Nathaniels (1996) scoring system as indicated before.

Overall scores on panicles and growth parameters were estimated as percentages and angular transformation (Arcsine) was carried out (Gomez & Gomez, 1984; Masawe *et al.*, 1997) for analysis of variance, except for the data on length of the germ-tube. Finally, all data were analysed using the ANOVA test in the statistical software MSTAT version 1.41, University of Michigan, U.S.A. Wherever justifiable, Duncan's multiple range test at $p \leq 0.05$ was used to compare and rank treatment means accordingly.

RESULTS

In total, 72 isolates (40 bacteria and 32 fungi) from cashew leaves and florets were tested as antagonists against cashew powdery mildew. None of these newly isolated potential antagonists were significantly effective in inhibiting disease development on panicles ($p = 0.3414$). Data from a 10 treatment experimental set are presented in Fig. 5.1.

On leaf discs, none of the commercial potential antagonists effectively prevented conidial germination in contrast to the standard fungicides, which significantly ($p = 0.0025$) reduced the percentage of germinated conidia (Fig. 5.2). *Bacillus licheniformis* and *B. subtilis* were as effective as the standard and recommended fungicide (triadimenol) in reducing the length of the primary hyphae ($p = 0.0253$) (Fig. 5.3). The yeast *C. saitoana* was less effective than the others but better than the negative control (water) (Fig. 5.3). However, all commercial biocontrol agents were significantly more effective in reducing the percentage of conidia with two or more hyphae as compared to the negative control (water) ($p = 0.0269$) (Fig. 5.4).

DISCUSSION

Previous studies reported the recovery of numerous isolates from the phylloplane of different plant species (Dickinson, 1976; Koomen & Jeffries, 1993; Korsten *et al.*, 1995; De Jager, 1999). The variation in types and numbers isolated is reflected in the technique used which varies between sampling unit, frequency in terms of time and space and more importantly the host and isolation technique chosen (Warren, 1976; Jacques & Morris, 1995). Through this investigation we recovered 72 isolates including bacteria and fungi, from cashew leaf and florets. The procedure was restricted to washable and culturable microorganisms which had been associated with the host / pathogen system during the sampling period. The aim of this strategy was to specially select potential hyperparasites since Casuli (1979) observed that *C. cesatii* was frequently associated with powdery mildew and he speculated that this hyperparasite could be a source of potential antagonism. However, in this study hyperparasitism was not investigated. This is the first report in which microbial isolates from cashew leaves and florets were isolated and evaluated against powdery mildew.

An important attribute of a successful biocontrol agent is the ability to be efficient at low concentrations (Korsten *et al.*, 1995). Thus, various concentrations ranging from 10^3 to 10^9 cells/ml have been used in evaluating potential antagonists against bacterial and / or fungal plant pathogens (Pusey & Wilson, 1984; Pusey, 1989; Korsten *et al.*, 1991; Korsten *et al.*, 1995; Dik *et al.*, 1998). Koumaki *et al.* (2000) succeeded in controlling cucumber powdery mildew (*S. fuliginea*) with bacteria and fungi at a concentration of 10^9 cells/ml while Dik *et al.* (1998) used only 10^6 cells/ml to reduce the level of the disease. Various authors working with bacterial antagonists commonly apply them at 10^7 cells/ml (Pusey, 1989; Pruvost & Luisetti, 1991; Korsten *et al.*, 1992). In our study, a concentration of 10^6 cells/ml of fungal antagonists and 10^7 cells/ml for bacterial antagonists were used, therefore within the commonly used range to effect.

A longer stabilisation period for the potential antagonist within the host plant organ is important for its preemptive colonisation and subsequent effectiveness against the pathogen (Fokkema, 1976). Our potential antagonists were added to the host only one day before inoculation with the pathogen. This takes into consideration the fact that production of susceptible tissue is continuous and prolonged for cashew (Shomari & Kennedy, 1999) and

the pathogen is polycyclic (Agrios, 1988). Therefore, simultaneous host colonisation is almost certain under field conditions. Thus, any promising antagonist must be a fast coloniser to be successful against powdery mildew. This may explain why bioagents were not as effective as chemical molecules in our experiment.

None of the potential biocontrol agents tested significantly reduced the percentage of conidia germination as compared to classic fungicides. But the initial growth stages of hyphae such as elongation and branching, were significantly reduced within 24 hours post inoculation with *C. saitoana, B. licheniformis* and *B. subtilis*. A comparable study where grape powdery mildew (*Uncinula necator* (Schwein)) was challenged with a chemical fungicide (pencanazole) at low concentrations also resulted in no inhibition of conidial germination but prevented hyphal development (Leinhos *et al*., 1997). This finding could be attributed to the fact that germ-tubes are more susceptible to environmental changes than conidia (Nathaniels *et al*., 1993). In conclusion, the above biocontrol agents could be established *in vivo* as potential antagonists of *O. anacardii*. Amongst these, *B. licheniformis* appears to be the most promising. It reduces both the growth in length and the branching capabilities of the primary hyphae. *B. subtilis* has successfully been used for field applications on various crops against various diseases (Korsten *et al*., 1997), but the fact that *B. licheniformis* isolates originally were from mango, which is a taxonomically close relative to cashew and has previously shown potential against mango mildew, may have contributed to its success in this experiment. However, growth and survival on cashew blossoms and leaves will require further testing under field conditions. Since endophytes live in an environment protected against sudden weather changes and radiation and are especially important for biological control (Tronsmo, 1992) further isolation work will be needed and perhaps semi-commercial screening.

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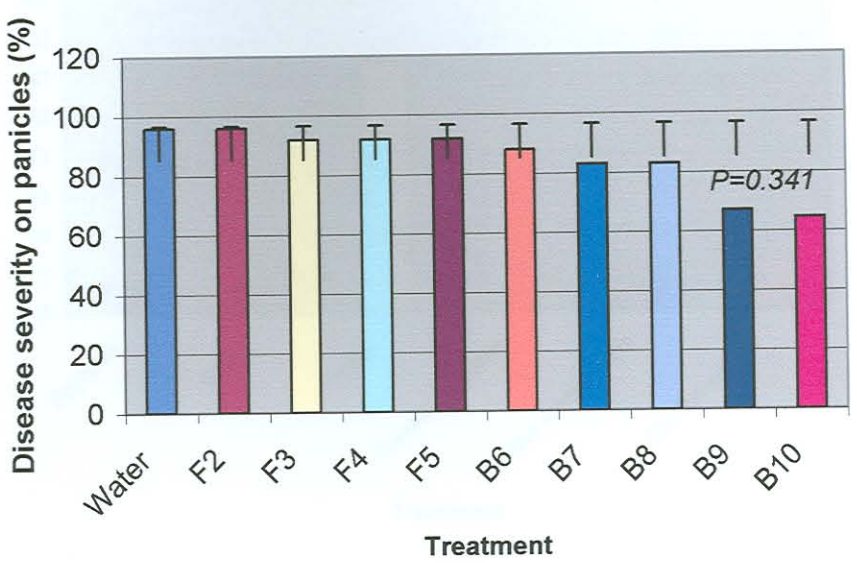

Figure 5.1 Powdery mildew severity means (in %) on panicles, 10 d after inoculation and 24 h later challenged with nine potential antagonists isolated from cashew trees and a negative water control. F = fungal antagonist, B = bacterial antagonist. Analysis of variance of angular (arcsin) transformed data, indicated $p = 0.3414$.

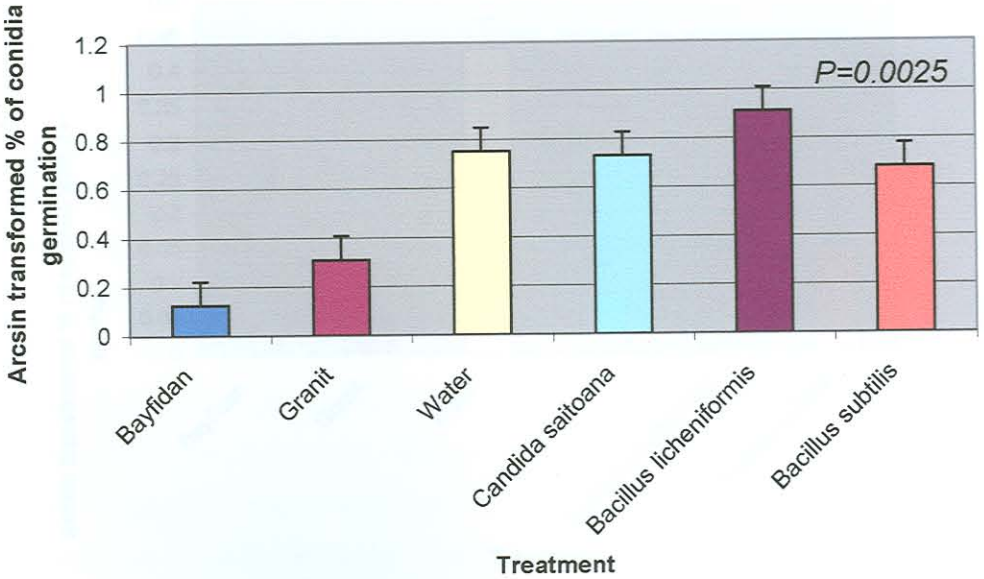

Figure 5.2 Effect of different treatments on angular arcsin transformed % of germinated conidia of *Oidium anacardii* Noack, applied 24 h prior to the pathogen inoculated on cashew leaf discs preserved on sorbitol 2% (w/v). Error bars represent the standard deviation of arcsin transformed % mean.

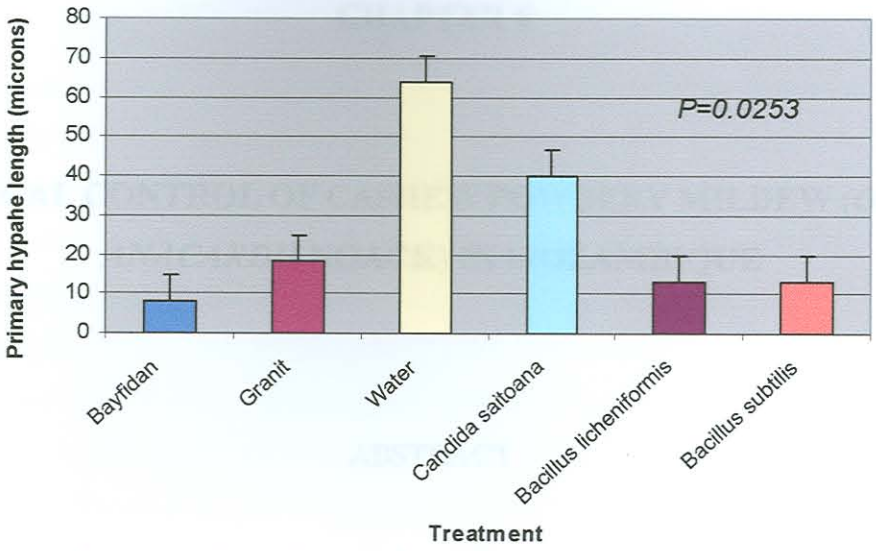

Figure 5.3 Effect of different treatments on the length at 200x magnification of the primary hyphae of *Oidium anacardii* Noack applied 24 h prior to the pathogen inoculated on cashew leaf discs preserved on sorbitol 2% (w/v). Error bars represent the standard deviation of mean lengths.

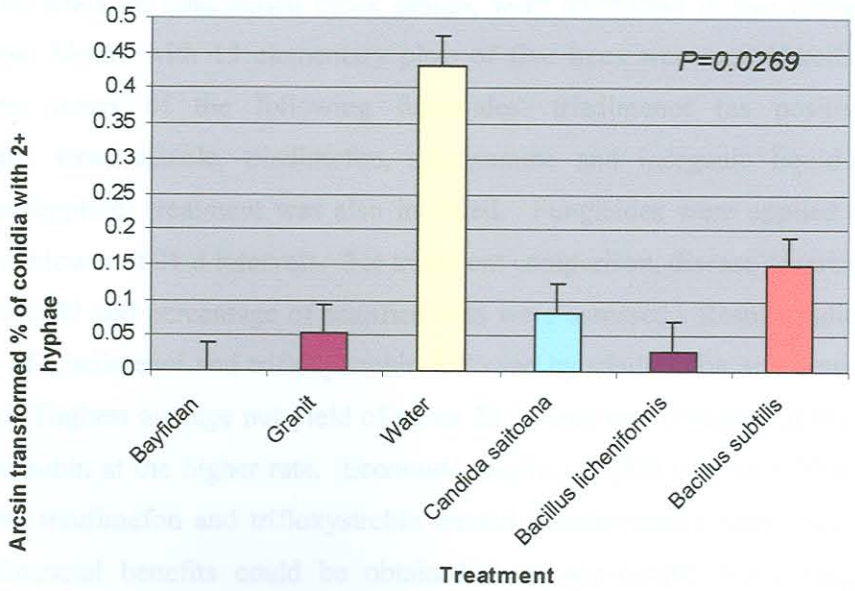

Figure 5.4 Effect of different treatments on angular (arcsin) transformed % of germinated conidia of *Oidium anacardii* Noack with two or more hyphae, applied 24 h prior to the pathogen which was inoculated on cashew leaf discs preserved on sorbitol 2% (w/v). Error bars represent the standard deviation of arcsin transformed % mean.

CHAPTER 6

CHEMICAL CONTROL OF CASHEW POWDERY MILDEW (*OIDIUM ANACARDII* NOACK) IN MOZAMBIQUE

ABSTRACT

Triadimenol and hexaconazole are the two fungicides commercially used to control powdery mildew (*Oidium anacardii* Noack) on cashew (*Anacardium occidentale* L.) in Mozambique from 1998. Being both triazoles, the risk of the pathogen developing resistance is high. In addition, cost-benefit analysis becomes important taking the economic dynamics of the country into account. Thus, on-farm trials were conducted with a view to assess biological and economical effectiveness of a series of chemical fungicides against cashew powdery mildew. Two trials, in randomised block design, were conducted in two consecutive crop seasons. Four blocks with 13 elementary plots of five trees were considered. Treatments included two doses of the following fungicides: triadimenol (as positive control), trifloxystrobin, tetraconazole, triadimefon, spiroxamine and inorganic liquid sulphur. A negative (non-applied) treatment was also included. Fungicides were applied three times, using a motor blower at 21 d intervals. For treatment comparison, disease severity on cashew panicles, nut yield and percentage of scarified nuts were assessed. Results indicated higher bio-efficacy of triadimenol and trifloxystrobin followed by triadimefon and liquid sulphur at higher doses. Highest average nut yield of about 23 kg/tree was obtained from plots treated with trifloxystrobin at the higher rate. Economic returns of USD 0.75 to 1.00 per tree were obtained from triadimefon and trifloxystrobin treated trees/orchard respectively. However, equivalent financial benefits could be obtained from non-treated plots, suggesting that fungicides in use at prevailing circumstances had no additional quantitative benefits to the farmers.

One of the cashew (*Anacardium occidentale* L.) production constraints in Mozambique since the 1970's has been powdery mildew (*Oidium anacardii* Noack) (Ohler, 1979; Dhindsa & Monjane, 1984; Milheiro & Evaristo, 1994; Nathaniels, 1996; Uaciquete; 1997). Today, yield reduction combined with industry policy related aspects, have led to more than 8000 direct job losses in Mozambique (Anon., 2003). To revert this scenario, integrated cashew powdery mildew management strategies are possible options. These include cultural practices such as sanitation, tree canopy size and shape modification either by pruning or top working susceptible plants with disease tolerant genotypes, field gap filling with tolerant material, and chemical control (Boma *et al.*, 1998; Maddison *et al.*, 1998; Waller *et al.*, 1992; Topper *et al.*, 2000). No information on biological control against cashew powdery mildew is available. Attempts of using biological control agents were made, but showed to be less effective when compared to chemicals such as Bayfidan for instance (Chapter 5). Furthermore, the viability of biocontrol product storage, and use for small-scale growers appears to be complicated.

An increase in the annual production of raw nuts in Tanzania has been achieved, mainly due to widespread use of sulphur to control the disease (Sijaona & Mansfield, 2001). Sulphur is a multisite inhibitor that interferes with electron transport along the cytochromes of the fungi (Delp, 1980; Agrios, 1988). In addition to sulphur, a number of systemic fungicides which operate as sterol biosynthesis inhibitors such as Bayfidan (triadimenol), Anvil (hexaconzole) and topas (penconazole) have been tested and recommended in Tanzania (Sijaona & Mansfield, 2001). Although sulphur appears to be of comparatively low cost and requires no water for application, its sustained use has been shown to cause soil acidification (Smith *et al.*, 1997). This is why sulphur was not adopted for cashew powdery mildew control in Mozambique. Currently, only Bayfidan and Anvil are in widespread use. However, both mentioned fungicides are triazoles, which pose a risk of potential build up of pathogen resistance. Site-specific inhibitors act on one or two metabolic sites and pathogen resistance is more common (Delp, 1980). On the other hand, a new generation of synthetic antifungal compounds (strobilurins), which inhibit mitochondrial respiration of the fungi by blocking the electron transfer at the cytochrome bc1-complex, has emerged (Leinhos *et al.*, 1997). Thus, the objective of this work was to screen chemical fungicides for powdery mildew disease control including a cost-benefit analysis of the spray program.

Two trials were conducted in the Northern region of Mozambique for the crop season 2001/2002 at Itoculo, Monapo District and 2002/2003 at Nassuruma (Salimo area), Meconta District. The layout of the experiments was a complete randomised block design (Gomez & Gomez, 1984) with three and four blocks respectively for Monapo and Meconta Districts. Five trees per plot were assigned per treatment. Trees at Monapo were about five years old, vegetatively propagated but heterogeneous in shape and size and subjected only to normal cultural practices such as cleaning and pruning. At Meconta District, cashew trees were established by seed, and were more than 20 years of age and subjected to similar crop management systems as those at Monapo. At both sites, natural rainfall was the only way in which trees were watered.

Trees were sprayed with commercial concentrations of different fungicides (Table 6.1) over a 21 d interval, starting July 22 for the 2001/2002 crop season and July 16 for the 2002/2003 season. Chemical treatments were applied with a water-based motorised mist blower (Solo) at a rate of 1.0 l/tree (Topper *et al.*, 2000). In both trials, a non-treated control was included to monitor the appearance of disease. The severity of powdery mildew on the control panicles (that is, no fungicides and no water applied) was not significantly different from that on the panicles which were treated with water (Masawe *et al.*, 1997).

As shown in Table 6.1 for the 2002/2003 crop season, the number of treatments increased due to integration of other potential chemicals and the discontinuation of Stroby due to lack of supply.

In both experiments, fungicide applications were initiated at the critical point of 15% panicle emergence and 10% panicle diseased as determined by the scouting quadrat method described by Boma *et al.* (1998). Inflorescence disease severity was assessed using a six grade scale (0, 5%, 25, 50, 75 and 100%) representing different levels of mildew infection (Nathaniels, 1996) as determined at the beginning of nut set and the second assessment, three weeks later (Boma *et al.*, 1998; Topper *et al.*, 2000).

Table 6.1 Chemical treatments per cashew crop season trial

Crop Season	Treatment	Formulation	Active ingredient	Chemical family	Rate (l/tree)
	Bayfidan	250EC	Triadimenol	Triazole	10.0 ml
2001/2002	Flint	500WG	Trifloxystrobin	Strobilurine	3.0 g
	Flint	50WG	Trifloxystrobin	Strobilurine	4.5 g
	Prosper	500EC	Spiroxamine	Spiroketalamine	15.0 ml
	Prosper	500EC	Spiroxamine	Spiroketalamine	20.0 ml
	Stroby		Kresoxim-methyl	Strobilurine	3.0 g
	Stroby		Kresoxim-methyl	Strobilurine	4.5 g
	Bayfidan	250EC	Triadimenol	Triazole	10.0 ml
	Bayfidan	250EC	Triadimenol	Triazole	15.0 ml
	Flint	50WG	Trifloxystrobin	Strobilurine	3.0 g
	Flint	50WG	Trifloxystrobin	Strobilurine	4.5 g
	Trical	250EC	Triadimefon	Triazole	10.0 ml
	Trical	250EC	Triadimefon	Triazole	15.0 ml
	Solfo Li	65%	Inorganic sulphur		100.0 ml
2002/2003	Solfo Li	65%	Inorganic sulphur		150.0 ml
	Eminent	40EW	Tetraconazole	Triazole	10.0 ml
	Eminent	40EW	Tetraconazole	Triazole	15.0 ml
	Prosper	500EC	Spiroxamine	Spiroketalamine	15.0 ml
	Prosper	500EC	Spiroxamine	Spiroketalamine	20.0 ml

Three different parameters were selected to determine the effectiveness of different fungicides and dose sprays: disease severity on panicles (Nathaniels, 1996), yield per canopy ground cover area (cgca) (Behrens, 1996; Topper *et al.*, 2000; Maddison *et al.*, 1997; Maddison *et al.*, 1998) and percentage of non-scarified nuts (clean nuts). In order to determine the percentage of clean nuts, a sample of 100 g of nuts per tree were collected at the beginning, middle and end of the harvesting season. The sampled nuts were separated into five categories of weight percentage: 0% scarified or clean nuts, 0-25% scarified nuts; 25-75%, 75-99% and 100% scarified (Fig. 6.3). Only the proportion of clean nuts was statistically analysed and presented in this report. Other categories which could not show significant differences between treatments were not processed for treatment comparison. The three parameters were chosen because they enabled assessment of the disease impact at three levels of cashew development:

Prior to nut formation, quantity and quality of produced nuts. Therefore, the parameters selected were complementary to one another.

Analysis of variance was performed for disease severity scores for each date and side of the tree, and the yield per cgca and transformed percentages (arcsin) of clean nuts were compared using Duncan's multiple range or Tukey's tests at 0.05 probability level.

Cost-benefit analysis was performed using a simplified model (Topper *et al.*, 1999), which encompassed the cost of fungicide, petrol and oil for the mist blower, depreciation of the blower per application and labour costs for spraying (Table 6.2). Harvesting and seasonal management costs were not included. On the other hand, cashew apple and other tree benefits are excluded from this evaluation. The total margin of benefit was calculated subtracting the total cost of fungicide application from nut yield (kg) times the price per unit.

Table 6.2 A model of cost benefit analysis for spraying a cashew tree three times during the season

Item	**Quantity used/application**	**Cost/unit (USD)**	**Cost/tree/season**
Fungicide	X[a]	X[a]	X[a]
Petrol (l)	0.05	0.5156	0.02578
2 Times engine Oil (l)	0.002	4.0469	0.0081
Depreciation of blower/ application		0.08	0.08
Labour costs		0.05	0.05
Total Cost			Y

a = Variable, dependent on chemical ; Source: Topper *et al.* (2000).

RESULTS

None of the treatments were toxic to cashew trees. Fungicide treatment differences were not detected for control of cashew powdery mildew when data from two different sides of the tree were compared at each date of observation for the crop season 2001/2002 (Table 6.3). Similarly, means of yield per area of canopy ground area coverage from all fungicide sprayed treatments were not significantly different, P>0.5779 for 2001/2002 crop season at Monapo District (Table 6.3). From this 2001/2002 trial, it is evident that the percentage of clean nuts from Stroby treated plots was significantly higher (70.6%) than that obtained from Flint 4,5 g/tree/application and Prosper 15 ml/tree/application treated plots, (Table 6.3).

For the 2002/2003 crop season, powdery mildew severity in both assessments and sides of the tree were significantly higher on untreated (negative control) plots than on treated ones (Table 6.4). Disease severity on Flint and Trical high dose treated plots was not statistically different from Bayfidan (positive control) sprayed plots (Table 6.4). The northern side of the tree, in both assessments, showed no statistical differences for all fungicide treated plots (Table 6.4). As in the previous season, yield means per area of canopy ground coverage from all fungicide sprayed treatments were not significantly different, P>0.0757 for the 2002/2003 crop season in the Meconta District (Table 6.4). For the percentage of clean nuts, treatment means were statistically different P>0.0057 (Table 6.4). The highest percentage of clean nut (25.5%) was obtained from plots sprayed with Flint 3g/tree/application. But it was not significantly higher than that obtained from other treatments (Table 6.4).

Chemical treatments on young trees at a rate of 1 l/tree showed to be uneconomical except for Flint, 3 g/tree per application and Prosper 20 ml/tree/application, which resulted in a small marginal benefit (less than USD 0.21 per tree). No treatment resulted in a much higher economic benefit to the farmer than that obtained from untreated plots (about USD 0.63 per tree) (Fig. 6.1). However, the use of fungicides on adult trees, at Meconta district, 2002/2003 crop season, resulted in economic benefits ranging from USD 0.21 to 0.83 per tree. The fungicide Prosper showed to be uneconomical at both dosages, while liquid sulphur (Solfoli) was uneconomical at 150 ml/l (Fig. 6.2). For Trical, Tetraconazole and Flint treatments an increased dosage resulted in a respective increase in economic benefits. However, this tendency was contrary for Bayfidan and Solfoli (Fig. 6.2).

Table 6.3 Performance of fungicide spray programs at 21-day intervals for control of cashew powdery mildew (*Oidium anacardii* Noack) during the 2001/2002 crop season in Monapo District, Mozambique

	Yield (g/m^2)	Powdery mildew severity (%)		Proportion of clean nuts (%)
		First assessment		
Tree side		North	South	
Grand Mean		7.3 (0.235)	14.6 (0.362)	
CV(%)		60	46	
Control Means		39.3	46.3	
Treatments				
Bayfidan 10		18.2 (0.412)	20.7 (0.465)	
Flint 3		12.5(0.351)	24.7 (0.497)	
Flint 4.5		7.7(0.253)	7.8 (0.265)	
Prosper 15		3.9(0.194)	16.0 (0.411)	
Prosper 20		4.9(0.197)	18.5 (0.392)	
Stroby 3		3.4(0.164)	9.4 (280)	
Stroby 4.5		0.8(0.071)	5.2 (0.223)	
Ftreat:Prob>F		0.1415 NS	0.3573 NS	
FBloc:Prob>F		0.8816 NS	0.2317 NS	
		Second assessment		
Grand Mean	116.3	2.1 (0.100)	14.8 (0.353)	56.8 (0.854)
CV(%)	25	89	59	10
Control Means	56.3	64	72.7	36
Treatments				
Bayfidan 10	142.2	6.7 (0.202)	24.4 (0.484)	63.4 (0.921 ab)
Flint 3	119.6	2.5 (0.141)	28.0 (0.549)	61.4 (0.901 ab)
Flint 4.5	101.6	0.9 (0.089)	7.9 (0.257)	47.8 (0.762 b)
Prosper 15	125.4	0.3 (0.030)	7.1 (0.264)	41.3 (0.696 b)
Prosper 20	102.7	3.3 (0.162)	19.7 (0.378)	54.5 (0.831 ab)
Stroby 3	103.7	0.9 (0.076)	7.0 (0.250)	58.5 (0.871 ab)
Stroby 4.5	119.2	0.00 (0.000)	9.7 (0.292)	70.6 (0.998 a)
Ftreat:Prob>F	0.5779 NS	0.1448 NS	0.4691 NS	0.0120*
FBloc:Prob>F	0.0042**	0.0680 NS	0.3761 NS	0.8816 NS

Legend of Fisher Test: NS = Non-significant at 5%; (* ** ***) significant at 5%, 1%, 1%0).

Means followed by the same letter do not differ significantly by Tukey's test at P = 0.05; numbers in ( ) are transformed data.

Table 6.4 Performance of fungicide spray programs at 21-day intervals for control of cashew powdery mildew (*Oidium anacardii* Noack) during the 2002/2003 cashew season in Meconta district, Mozambique

	Yield (g/m^2)	Powdery mildew severity (arcsin transformed %)		Proportion of clean nuts (%)
		First assessment		
Tree side		North	South	
Grand Mean		0.097225	0.1911	
CV(%)		138.8	98.0	
Control Means		0.670 a	0.9918 a	
Treatments				
Bayfidan 10		0.01532 b	0.323 c	
Bayfidan 15		0.00075 b	0.0093 c	
Flint 3		0.00175 b	0.0451 c	
Flint 4.5		0.02552 b	0.0594 c	
Trical 10		0.02998 b	0.0634 c	
Trical 15		0.00972 b	0.0322 c	
Solfoli 100		0.12054 b	0.1861 bc	
Solfoli 150		0.01824 b	0.1026 c	
Eminent 10		0.19504 b	0.2550 bc	
Eminent 15		0.00650 b	0.0986 c	
Prosper 15		0.08306 b	0.4075 b	
Prosper 20		0.8692 b	0.2011 bc	
Ftreat:Prob>F		0.0001***	0.0001***	
Fbloc:Prob>F		0.1140	0.0097	
		Second assessment		
Grand Mean	107.2	0.2174	0.434128	14.2 (0.356)
CV(%)	65	86	59.39	35
Control Means	75.5	1.2738 a	1.3909 a	4.1 (0.158)
Treatments				
Bayfidan 10	113.7	0.0157 b	0.0884 de	14.6 (0.356 ab)
Bayfidan 15	140.1	0.0055 b	0.0235 e	23.3 (0.500 a)
Flint 3	165.5	0.0110 b	0.1747 cde	25.5 (0.517 a)
Flint 4.5	184.1	0.0310 b	0.1110 de	20.8 (0.470 ab)
Trical 10	55	0.2225 b	0.4686 bcd	6.2 (0.243 ab)
Trical 15	82.1	0.1363 b	0.3210b cde	9.1 (0.268 ab)
Solfoli 100	68.8	0.2210 b	0.5543 bc	13.6 (0.338 ab)
Solfoli 150	190.7	0.0589 b	0.3218b cde	18.9 (0.417 ab)
Eminent 10	69	0.2505 b	0.6318 b	10.2 (0.315 ab)
Eminent 15	104.9	0.0782 b	0.3158b cde	18.6 (0.436 ab)
Prosper 15	73.2	0.3068 b	0.5929 bc	12.8 (0.359 ab)
Prosper 20	70.9	0.2156 b	0.6492 b	6.7 (0.255 ab)
FTreat:Prob>F	0.0757 NS	<0.0001***	<0.0001****	0.0057 **
Fbloc:Prob>F	0.3137 NS	0.0160	0.0003	0.0032 **

Legend of Fisher Test: NS = Non significant at 5%; (* ** ***) significant at 5%; 1%, 1%0)

Means followed by the same letter do not differ significantly by Duncan's (powdery mildew severity) or Tukey's test at P = 0.05; numbers in brackets are transformed data.

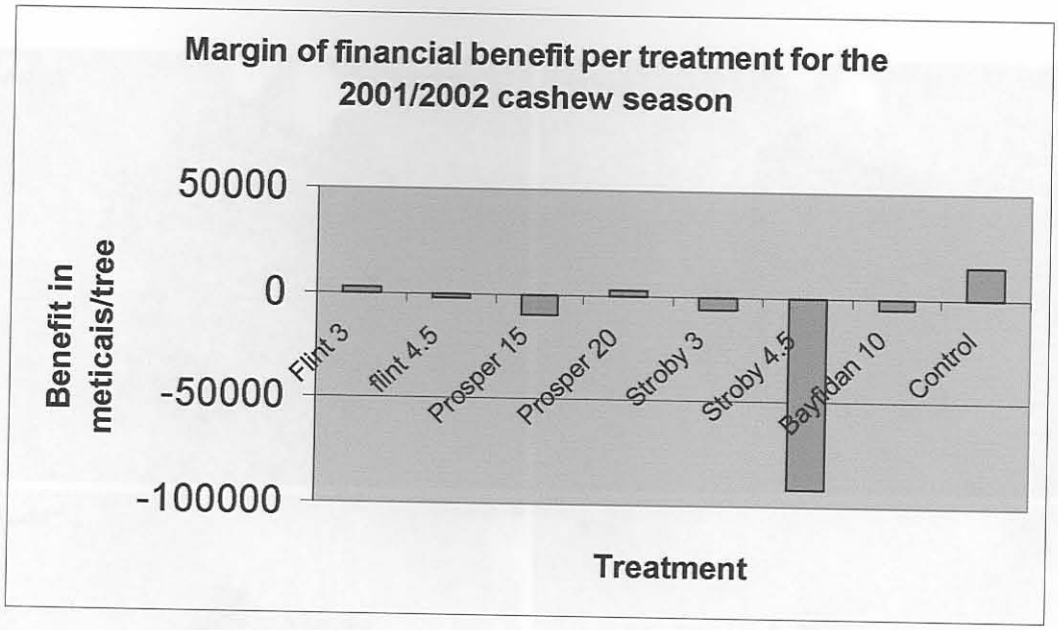

Figure 6.1 Treatment effects after fungicide spray programs for the control of cashew powdery mildew (*Oidium anacardii* Noack) during the 2001/2002 crop season in Monapo district, Mozambique; 24 000.00 Meticais = 1 USD.

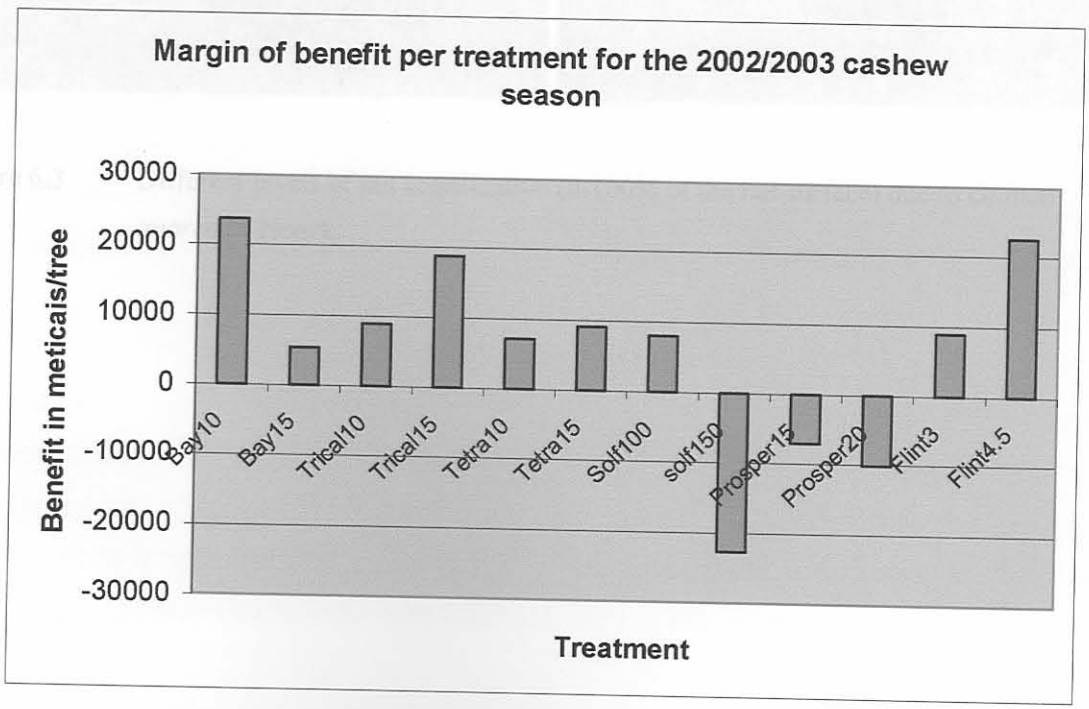

Figure 6.2 Treatment impact of fungicide spray programs for the control of cashew powdery mildew (*Oidium anacardii* Noack) during the 2002/2003 crop season in Meconta district, Mozambique; 24 000.00 Meticais = 1 USD.

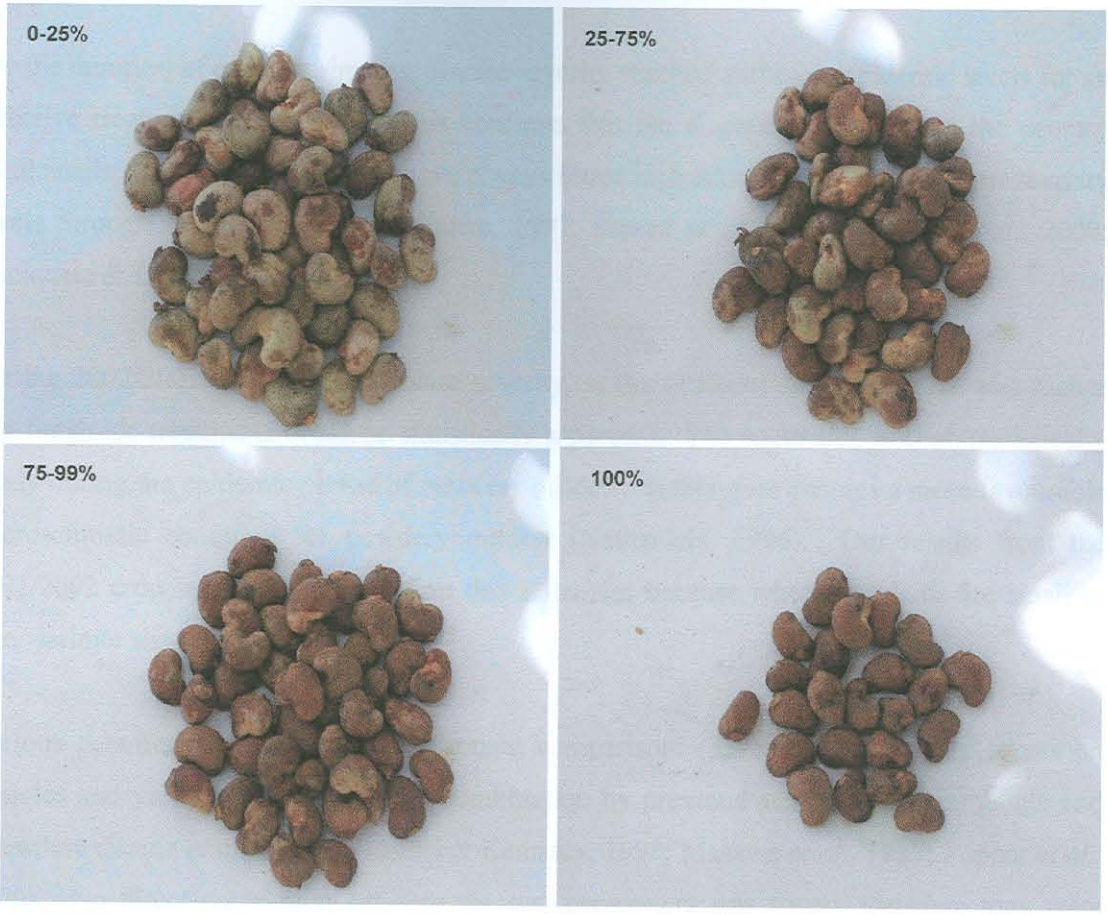

Figure 6.3 Different levels of nut scarification (0-100% of the nut surface) due to *Oidium anarcadii* Noack.

DISCUSSION

For the duration of our experiments, disease severity reached sufficient epidemic levels for an effective treatment comparison. This confirms that the disease is endemic in the country (Nathaniels, 1996). Other workers have always found high levels of powdery mildew severity levels throughout the country (Uaciquete, 1997; Prasad *et al.*, 2000; Topper *et al.*, 2000; Uaciquete & Lyannaz, 2002a,b).

For the 2002/2003 crop season, disease severity on the southern side of the tree was higher and fungicide treatments could be statistically separated. The south side is characteristically shady during the epidemic period of powdery mildew. It therefore ensures a more favourable micro-climatic condition for powdery mildew (Nathaniels, 1996). Our results from the 2001/2002 crop season cannot confirm this statement because when the plants are small in size, definite shady sides do not exist.

Various parameters were used for treatment comparison: Two disease severity scores on panicles and yield have been used in combination by previous authors in Mozambique and elsewhere (Boma *et al.*, 1998; Shomari & Kennedy, 1998; Masawe *et al.*, 1997; Topper *et al.*, 2000). No direct correlation between the two parameters was found, therefore they were considered to be complementary to one another (Boma *et al.*, 1998). In this experiment, a third parameter of nut quality (% of clean nuts) was introduced, providing additional information on assessing the impact of fungicide treatment on the marketable product.

For the most effective fungicides, disease severity did not reach beyond 10%, while untreated plots showed more than 90% damage on the panicles. The findings are in accordance with previous studies: In 14 trials conducted on-farm, Topper *et al.* (2000) obtained less than 30% disease severity with Bayfidan or Anvil treated plots against 80 to 90% damage on untreated plots. Similarly, Gibberd (2002) observed, with a fungicide testing trial at Monapo district, a maximum of 20% disease severity with fungicides compared to 90% on untreated cashew.

Recommended minimum dose for sulphur application against cashew powdery mildew is 125 g a.i./tree/spray (Topper *et al.*, 1999; Topper *et al.*, 2000). In our trial, liquid sulphur (65%) was applied at maximum dose of 150 ml/tree/round. This implies that only 97.5 g of active ingredient was deposited per tree/round.

Average nut yield per tree on untreated plots were 2.4 kg at the Monapo trial and 4.0 kg at Salimo. National nut yield average from untreated plots is between 3 and 4.3 kg (Machungo, 2002). Our results are therefore in accordance with other findings.

Working with systemic fungicides (Anvil and Bayfidan) Topper *et al.* (2000) obtained an average of 27 kg/tree from sprayed plots. Our results at Salimo indicated a range between 5 and 23 kg/tree depending on the dosage and fungicide applied. Bayfidan treated plots increased yield from 4 to 10 kg/tree. Topper's data were estimated as nut counts on the tree and not mature harvested nuts. This may explain the difference of nut yield average on Bayfidan treated plots. Extensive use of systemic fungicides on farmer's fields has increased yield from 3 to 12 kg/tree (Machungo, 2002). This finding appears to be more realistic, although low efficacy may be associated with inefficient use by extension workers.

High yields obtained from Bayfidan (triadimenol) and Flint treated plots may be related to the effect of both chemicals against cashew, and anthracnose (*Colletotrichum gloeosporioides* Penz.) (Smith & Manicom, 1999; Freire *et al.*, 2002) which may occur in conjunction with powdery mildew (Lopes *et al.*, 1993).

Percentage of clean nuts from Flint and Bayfidan treated plots suggest that there are no direct relationships between this and the severity of disease on panicles. It is possible the genetic precocity of some cashew clones and timing of applications may have higher influence on the percentage of clean nuts harvested than severity of the disease on panicles.

From our findings, the percentage of clean nuts on fungicide treated plots is consistently higher than on untreated ones. This supports the statements by Topper *et al.* (2000) who analysed the kernel turn-out from both treated and untreated nuts and found a significant reduction on turn-out of about 22% from diseased nuts, thus affecting the quality of nuts.

An increase in the rates of Flint, Trical and Eminent resulted in corresponding increases on yield and thus the benefit of USD 2.5, 0.5 and 0.083 respectively. This suggests that more trials with an increased dose for Flint and Trical would be necessary before a final recommendation.

In our experiment, the highest margin of economic benefit was USD 1.04, 1.00 and 0.75 per tree for Bayfidan (10 ml); Flint (4,5 g) and Trical (15 ml) respectively. These results are

surprisingly low. Prior to our study, Topper *et al.* (2000) estimated the benefit from the use of Bayfidan or Anvil to be between USD 9 and 12 per tree. The author over-estimated the price of nuts (USD 0.5/kg) against the current USD 0.24/kg and under-estimated the cost of organic fungicides USD 32/l against the actual USD 37/l for Bayfidan. In addition, the author based his calculation on the potential yield estimated by nut counting, a method that may have over-estimated the yield. The nut counting method used in this study was especially developed because nuts were frequently stolen from the experimental plots. No cases of nut stealing or hiding were reported from our study.

Our study has highlighted the low benefits that farmers get from the use of systemic fungicides in general (USD 0.75-1). In fact, such yield benefits could also be obtained without the use of fungicides. Approximate value (USD 0.96) could be obtained from untreated plots yielding 4kg/tree on average.

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CHAPTER 7

GENERAL DISCUSSION

Agriculture began, probably 8 000 to 10 000 years ago, somewhere between the Nile in Egypt and the valley of the Indus River in western India (Salmon & Hanson, 1964). Since then, mankind has been challenged with crop diseases (Baker & Cook, 1974). However, only around the middle of the nineteenth century, control of diseases began to receive special attention (Salmon & Hanson, 1964; Agrios, 1988). Today, control of plant diseases is most successful and economical when all available pertinent information regarding the crop, its pathogens and the environmental conditions are part of an integrated control program. This may comprise quarantine, evasion of the pathogen, improving the growing conditions of plants, various cultural and sanitation methods, the use of fungicides, biological control agents and the use of resistant varieties (Agrios, 1988). Integrated control programs are well known for crops such as citrus and mangoes (Agrios, 1988; De Jager, 1999) and cashew (*Anacardium occidentale* L.) is no longer an exception (Waller *et al.*, 1992; Martin *et al.*, 1998).

The present study was aimed at elucidating aspects of host tolerance mechanisms to the powdery mildew pathogen (*Oidium anacardii* Noack) infection, disease epidemiology and pathogen reaction to potential antagonists and chemical fungicides with a general view of integrated disease management.

Cashew was introduced into east Africa in the XVIth century (Milheiro & Evaristo, 1994) but economically important phytopathological aspects were only recorded from the 1970's onwards (Ohler, 1979; Intini, 1987; Milheiro & Evaristo, 1994). Amongst the various diseases reported, powdery mildew was the most important in Tanzania and Mozambique (Nathaniels, 1996). A wide range of options for integrated cashew management (ICM) has since been evaluated with limited success (Waller *et al.*, 1992; Martin, *et al.*, 1998; Shomari, 1998; Topper *et al.*, 2000). This study illustrated the mechanism of infection of *O. anacardii* on both susceptible and tolerant cashew varieties. Host type reaction differences based on cytoplasmic aggregate densities were noted. The enormous increase in cytoplasm below the infection site seemed to result mainly from synthesis (McKeen & Rimmer, 1973) or rapid

traumatotactic migration of cytoplasm (Bushnell & Zeyen, 1976). Similar results have been associated with expression of partial resistance in other host-pathogen systems such as *Puccinia graminis* Pers. on wheat, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. on beans and *Phytophthora infestans* (Mont.) de Bary on potato (Bushnell & Zeyen, 1976; Aist & Bushnell, 1991). The applicability of the finding in terms of general identification of varietal cashew tolerance to powdery mildew was not explored further.

The present study confirmed that *O. anacardii* possesses a short conidiophore, with one basal cell (Castellani & Casulii, 1981) and that it produces ellipsoidal, thin walled, unicellular and hyaline conidia (Shomari, 1996). It also revealed that the conidial outer wall pattern is honey-comb ornamented with predominantly smooth septa. Such characteristics fit in well for members of *Oidium* subgenus *Pseudoidium* (Y.S. Paul & J.N. Kapoor) comb. Et. Stat. Nov. (Holomorph *Erysiphe* Sect. *Erysiphe* U. Braun) of the new classification proposed by Cook *et al.* (1997). However, it should be noted that the perfect stage of *O. anacardii* is still unknown (Intini, 1987; Shomari & Kennedy, 1998).

When the atmospheric relative humidity, at temperatures of 26-28°C, is around 90-100% for at least six hours, *O. anacardii* is capable of infecting the host (Intini, 1987). However, for a powdery mildew epidemic to occur, weather conditions favourable for conidia release are necessary and susceptible tissue must be available (Schoeman *et al.*, 1995). We found susceptible cashew inflorescences blooming from May to November. This coincides with previous observations by Milheiro and Evaristo (1994). However, the appearance of cashew powdery mildew epidemics was only determined by conditions of average minimum and maximum temperatures between 20 and 30°C, air relative humidity of at least 40% and an estimated dew point around 30, under the tree canopy. Temperatures below 15 and above 35°C are known to restrict conidial germination (Shomari & Kennedy, 1999) and thus may have played a major role for disease onset or decline respectively. However, the magnitude of changes of any of the above weather parameters during the course of the disease epidemic could not be directly related to fluctuations in disease severity.

Shomari (2000, *personal communication)* derived a predictive model using data from *in vivo* work which described the effect of temperature and relative humidity conditions on germination of *O. anacardii* conidia. However, the model could not succeed in the field due to variations in conducive conditions for conidial germination between the interior and

exterior of the tree canopy (Shomari & Kennedy, 1999). Localities have different powdery mildew development patterns and nut production ratios (Nathaniels & Kennedy, 1996; Topper *et al.*, 2000). A model formulated to monitor the disease progress as a function of climatic influences would enable a more precise timing of fungicide applications and thus avoid unnecessary sprays (Peak *et al.*, 1986). Therefore, the effect these and/or other weather parameters have on other processes such as sporulation rate and penetration processes needs to be explored.

The microbial antagonism that is seen in biological control of plant pathogens is broadly based on the categories of competition (for nutrient and space), parasitism (which may be production of volatile or nonvolatile antibiotics) and hyperparasitism (Singh & Faull, 1988). The 72 isolates from mildew infected cashew leaves and florets had no significant inhibition effect on conidial germination and mycelium development of *O. anacardii*. The pathogen-antagonist niche-overlap pre-condition for a viable biocontrol interaction (De Jager, 1999) was fulfilled at sampling as well as at screening. However, none of the natural epiphytes were antagonistic *in vivo* while the commercial products were. *Bacillus subtilis* and *B. licheniformis* were previously shown to be antagonistic against a range of plant pathogens on avocado (*Persea americana* Mill.) (Korsten *et al.*, 1995; Korsten *et al.*, 1997) and mango (*Mangifera indica* L.) (Pruvost, 1991). Mangoes, avocado and cashew all belong to the family Anacardiaceae. Stack *et al.* (1988) stated that some organisms may be effective only in the presence of other related microorganisms or additives. This is why some formulated products contain nutrients that enable bio-agents to survive during the initial establishment stages (Fokkema, 1976; Hirano & Upper, 1986). Furthermore, Klincare *et al.* (1971) studied the composition and activity of the epiphytic microflora of some Russian agricultural plants and concluded that under conditions of pure culture, only a few were in antagonistic interrelationship with each other. In this research, antagonist combinations were not investigated and should be considered in future studies.

The comparative study of fungicides illustrated that the use of triazoles for powdery mildew control on cashew in Mozambique can be integrated with other fungicides such as Trical or Flint. However, it was also demonstrated that under the current economic environment within Mozambique, none of the fungicides resulted in increased economic benefits compared to non-treated plots. A major understanding of integrated cashew management strategies (Waller *et al.*, 1992; Masawe, *et al.*, 1997; Boma *et al.*, 1998; Sijaona & Mansfield 2001;

Sijaona *et al.*, 2001) is therefore recommended in order to minimise the number of applications and maximise biological efficiency of the chemicals. Policy considerations on the price at farmer's level, based on the quality of nuts rather than simply quantity, need to be revised.

The present research brings together aspects of germplasm identification for tolerance to powdery mildew which, if properly integrated with the knowledge on disease epidemiology plus chemical and biological control, would enhance cashew nut production in Mozambique. From this study, future work should focus on determining economic integrated treatment programs that will reduce the costs involved in cashew nut production today.

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