Epidemiology of **cashew anthracnose** (***Colletotrichum gloeosporioides Penz.***)**in Mozambique**

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Abstract

Anthracnose of cashew (*Anacardium occidentale*) was studies on various genotypes and locations in Mozambique. *Colletotrichum gloeosporioides* was identified as the anthracnose causal agent using polymerase chain reaction. The relationships between incidence and severity of anthracnose on cashew genotypes were statistically analyzed by regression. Anthracnose leaf incidence, which is practically easy to evaluate, was consistently associated with leaf severity, and their relationships can be estimated using the restricted exponential function across locations, crop seasons, genotype and fungicide trials. Pooled data enabled estimation of initial incidence of 1.43% with percentage variance accounting for 83.2 and standard error of 8.3. By computing incidence data into the summary equation, 24 changes of 0, 1, 5, 10 and 40%, resulted in changes of severity estimates of 0.01, 0.05, 0.10, 0.50 and 1.00%, respectively. The maximum disease incidence was estimated as 80% when the severity reached only 5%. Increase in severity was observed afterward, approached a maximum of 25% when leaf detachment is observed. The use of incidence data for epidemic comparisons, genotype and fungicide evaluation in cashew orchards is recommended. Anthracnose incidence on leaves however, could not predict incidence on nuts.

Key words: Anacardium; nuts; epidemiology; disease assessment.

1. Introduction

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

Disease symptoms manifest in both leaves and young nuts (Freire et al., 2002). Severe damage on adult plants results in defoliation during shoot development, death of inflorescences and later necrosis and falling of immature nuts (Freire and Cardoso, 2003). Damage assessment can therefore be done at any of the above stages depending on the purpose of the evaluation, which can be fungicide (Da Matta and Lellis, 1973) or germplasm screening (Cardoso *et al*., 1999), or epidemiological investigations (McRoberts et al., 2003;Cardoso et al., 2004). In any of the above approaches, terminology such as disease incidence, disease severity, disease density and others are commonly used to measure the disease. Relative advantages and practical applications of their relationships have been discussed (McRoberts et al., 2003). Nevertheless, practical

limitations resulting from inconsistency of the relationships across locations, stage of the epidemic, host genotype and crop cycle have been found 49 (James and Shih, 1973; Rouse et al., 1981;Chuang and Jeger, 1987). Many other authors (Silva-Acuna et al., 1999) have however found simple, consistent and useful relationships in different pathosystems. Tedious and time consuming work associated with severity measurement has been replaced by the easily measured incidence (Silva-Acuna et al., 1999; Cardoso et al., 2004). In this study, PCR technique was used to establish the identity of the causal agent of cashew anthracnose and a standardized visual key (Nathaniels, 1996) was adopted with an objective to explore the use of the relationship between incidence and severity to comparatively characterize the development of cashew anthracnose epidemics across seasons, genotypes and locations. Furthermore, we explore the possibility of developing a predictive model for incidence on nuts based on incidence or severity on leaves produced before the setting of nuts.

2. Materials and methods

2.1. Pathogen identification

Anthracnose symptomatic samples were collected from all the trial sites and pathogen isolations made on potato dextrose agar (PDA). Fungal mycelia were then harvested from PDA cultures in laminar hood and total genomic DNA extracted using the DNeasy Plant Mini Kit (Qiagen Inc.) (Anon., 2004). For PCR amplification, species-specific primers from the ITS1 region of the ribosomal DNA gene (Ivey et al., 2004) CaInt2 (5'-GGC-GCCGGCCCCGTCACGGGGG-3') and CgInt (5'-GGCCTCCCGCCTCCGGGCGG-3') for *C. acutatum* and *C. gloeosporioides* respectively, were individually coupled with the universal and conserved primer ITS4 (Ivey et al., 2004; Whitelaw-Weckert et al., 2007). Amplification reactions were performed in an Epperdof Master Thermocycler (Merck Chemicals Pty Ltd, South Africa). Each reaction mix (microL) contained: 0,5microL DNA, 0.5 microL of 2.5 mM each dNTP, 1.25 microL of 50 mM MgCl2, 1x NH4 reaction buffer, 0.3 microL of each 1x diluted primer (24,20 nmol CaInt2; 25,30 nmol CgInt and 15 mg/l ITS4), 0.25microL of Taq DNA polymerase. Amplification cycles were as described by Ivey et al., 2004. PCR products (0.5 microL) were separated by horizontal gel electrophoresis in a Maxicell EC360M electrophoretic gel system (Electrophoretic gel system, EC Apparatus Corporation) coupled to a 250/2.5 voltmeter model (Bio-Rad, South Africa). One percent agarose gels were immersed on TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0)

at 100 V for 60 min. The gels contained 10 mg/ml ethidium bromide as stain. The DNA bands were visualized under UV light and photographed with the aid of Vilber Lourmat photosytem (Marne la Vallee, France).

2.2. Epidemiological studies

Cashew orchards were located in four sites of Northern Mozambique (Table 1). The plants were rain fed and cropping practices consisted of weeding and application of fungicides against powdery mildew.

Table 1. Characteristics of the trial sites in which cashew anthracnose incidence and severity data were collected in Mozambique during 2006 and 2007.

Distance from Nassurma (km)	GPS coordinates		Type of grafted cashew progenies	Quantity of cultivars & replicates	Plant spacing (m)	Plant age (years)	Owned by	Screening trial for
0			Dwarf progenies	10 & 3	8 × 6	9	The IIAM[a]	Germplasm
132			Mixed	Unknown & 4	8 × 6	7	A cashew farmer	Fungicide
460	37k029049	utm 8137405	Dwarf progenies	40 & 3	10 × 10	7	NGO[b]	Germplasm
460	37k029049	utm 8137405	Common progenies	33 & 3	10 × 10	7	NGO	Germplasm
512	37k0445057	utm 8139091	Dwarf progenies	67 & 3	10 × 10	8	INCAJU[c]	Germplasm
512	37k0445057	utm 8139091	Common progenies	80 & 3	10 × 10	8	INCAJU	Germplasm

[a] Agriculture Research Institute of Mozambique.
[b] Non-governmental organization.
[c] National Institute for Cashew Development.

Fungicides used were: Volcano Richter (hexaconazole SC 5%, Volcano AgroScience (Pty) Ltd) and Voltriad (Triadimenol SC 5%, Volcano AgroScience (Pty)) both at a rate of 10ml/L/tree of formulated product, three times a season at 21-day intervals (Sijaona and Mansfield, 2001). At Rapale, data were collected from a fungicide trial with weekly applications of hexaconazole.

At the beginning of each crop season, from both north and south sides of individual

trees, five shoots were tagged with a sisal cord of approximately 25 cm (Sijaona and Mansfield, 2001). Shoot development stages were 74 recorded by allocating discrete numbers from 1 to 9 at shoot burst and nut maturation respectively (Conticini, 1982).

New shoots are easily recognizable since they are greener and tender compared to older ones from the previous crop season.

The disease was assessed on a maximum of ten new leaves per crop season per shoot and for two consecutive crop seasons 2006 and 2007. Assessments began in May/June and ended in September according to the development and maturation of shoots. Incidence and severity were evaluated simultaneously based on the standardized leaf damage scale developed for cashew powdery mildew (Nathaniels, 1996). In this study, severity described the percentage of necroticised leaf area while incidence reflected the percentage of diseased leaves (McRoberts et al., 2003; Cardoso et al., 2004). Later in each crop season, anthracnose incidence on the nuts was also assessed as percentage of symptomatic immature nuts/panicle/plant. Disease scores were initially processed to return plant mean scores as detailed by Masawe et al., (1997).

For each individual crop season, the cashew phenology, incidence and severity data were tabulated in an excel spreadsheet per location, date of observation, replicate treatment and plant. In each location and cropping season, the progress of phenological stages of the crop was obtained by plotting means over treatment and plant against the date of recording.

Regression analysis of incidence and severity from un-transformed data were performed using GenStat (2003) package for windows. Variables means over date and treatment were computed to fit an exponential function (Snedecor and Cochran, 1980; Cornell and Berger, 1987). Incidence was the response variant 99 and severity the explanatory (McRoberts et al., 2003; Cardoso et al., 2004). Furthermore, leaf severity and incidence were used as explanatory to the incidence on nuts.

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

3. Results

3.1. Pathogen identification

The species specific primer CgInt coupled with the ITS4 primer successfully amplified the same size fragment from genomic DNA of all Colletotrichum isolates as the positive reference *C. gloeosporioides* isolate from mango. No additional band was observed closer to the reference (Fig. 1). No PCR-amplified product for primer CaInt2 was detected. Negative controls had no amplification product.

3.2. Epidemiological studies

Table 2. Regression equations of incidence (*I*) on severity (*S*) of leaf anthracnose (*Colletotrichum gloeosporioides*) under different environments and different cashew (*Anacardium occidentale*) genotypes in Mozambique, 2006–2007.

Location	Type germplasm	Year	*b*	*K*	*a* = (*K* + *b*)	*R*	Percentage variance accounted for	SE
Nassuruma	Dwarf progenies	2006	68.56	(−59.82)	8.74	0.8595	82.5	6.340
Nassuruma	Dwarf progenies	2007	123.00	(−120.00)	3.00	0.9404	92.5	3.420
Rapale	Dwarf & common	2007	24.2	(−23.6)	0.60	0.726	91.3	1.090
Mocuba	Dwarf progenies	2006	87.44	(−87.85)	(−0.41)	0.8650	74.5	9.860
Mocuba	Dwarf progenies	2007	76.91	(−75.46)	1.45	0.8056	84.8	6.950
Mocuba	Common Progenies	2006	100.7	(−99.3)	1.40	0.8775	82.5	7.710
Mocuba	Common progenies	2007	86.94	(−84.13)	2.81	0.8569	86.8	7.373
Pebane	Dwarf progenies	2006	78.71	(−79.06)	(−0.35)	0.765	68.2	14.200
Pebane	Dwarf progenies	2007	55.40	(−56.32)	(−0.92)	0.6608	79.8	9.380
Pebane	Common progenies	2006	88.26	(−87.83)	0.43	0.7980	84.6	13.100
Pebane	Common progenies	2007	85.22	(−86.24)	(−1.02)	0.7402	88.1	12.1
Overall mean			**79.58**	**(−78.15)**	**1.43**	**0.8086**	**83.2**	**8.320**

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

The relationship between incidence and severity on the cashew leaf anthracnose pathosystem was consistently best characterized by the restricted exponential function (1) across locations, crop seasons, cashew genotype or fungicide trials (Table 2).

$I = b\,(1-e^{-as})$ (P***<0.001) (1)

In this function, I stands for incidence, S for severity, *b* for estimated maximum incidence (emi) and *a* stands for estimated initial disease incidence (eii).

The potential for high epidemics in each location or crop season was expressed by the emi value (asymptote of the resctricted exponential curve) noting however that the explicit maximum is 100% (McRoberts et al., 2003). Thus, at Nassuruma germplasm screening trial, for the 2007 crop season the emi value was 123.5% (Fig. 2b, C)against 68.56% for 2006 (Fig.2a, A). Therefore year 2006 was less conducive to disease spread than the following crop season.

Estimated initial incidence (eii) in the restricted exponential function (Table 2) expresses the abundance of inoculum or minimum aggregation of the pathogen that is not visually recordable (McRoberts et al., 2003). In the cashew anthracnose pathosystem, such inoculum may derive from 123 nearby infected plants and mummified fruits. More predominantly inoculum may come from leaves of previous vegetative growth that anticipate the reproductive growth within a year. Thus, at Nassuruma, the eii value was higher (8.74%) in the 2006 crop season than 2007 (3.00%) (Table 2).

In contrast, at Mocuba, the year 2006 crop season showed a relatively higher emi value compared to year 2007 for both dwarf (Fig.3a, A and Fig.3c, E) and common types (Fig. 3b, C and Fig. 3d, G). At Mocuba and Pebane the eii values of both dwarf and common cashew type trials for the crop season 2006 were lower compared to year 2007 (Table 2). This suggests that in the same location, the abundance of viable initial inoculum varies from one season to another.

At the Mocuba trial on dwarf cashews the emi was 87.63% (year 2006) and 76.91% (year 2007), relatively lower than that of the common cashew trial (100.7% for 2006 and 86.94% for the year 2007 respectively) (Table 2). The eii on common cashew types was 1.4% in 2006 and 2.81 % in year 2007, both relatively higher than that on dwarfs, which were -0.41% and 1.45% for years 2006 and 2007 respectively. At Pebane, common cashew type trial, like Mocuba (Fig. 3b, C,D), the 2006 crop season was highly conducive to disease development (Fig.4 A,B).

At Rapale, during the 2007 crop season the lowest value of emi (24.18%) was observed (Fig. 5, A), however the incidence –severity relationship remained robust as an exponential curve. At Rapale, observed maximum incidence (12%) and maximum severity (2.4%), of anthracnose were relatively lower compared to Mocuba, Nassuruma and Pebane trial sites due to fungicides applications.

High emi values (disease spread) were consistently found in association with showers during the first week of July (Fig. 2a,b; Fig 3a to Fig. 3d, Fig.4 and Fig.5) regardless of location or crop season.

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

Using data from three locations (Rapale, Nassuruma and Pabane) the attempt to linearly

associate incidence on leaves to incidence on nuts formed later in the season was not significant (*P>0.001)* (data not presented).

anacardii Noack) (Dhindsa and Mondjana, 1984). Because, *Colletotrichum gloeosporioides* is species group, variable in morphology, pathogenicity and physiology (izoenzymes produced) (Freire and Cardoso, 2003). In the present study, *C. gloeosporioides* and *C. acutatum* were targeted by PCR-technique. However, no evidence of this last was detected. Therefore, cashew anthracnose in Mozambique was molecularly confirmed to be caused *C. gloeosporioides*. This is in conformity with findings from Brazil (Cardoso and Freire, 2002).

Numerous publications have dealt with the incidence-severity relationship of various pathosystems (Silva-Acuna, et al., 1999; Cardoso et al., 2004). Various models have been produced and their application and limitations were reviewed (McRoberts et al., 2003; Cardoso et al., 2004). In our study, the relationship between incidence and severity on cashew leaf anthracnose non-transformed data, best fitted the restricted exponential family model. This model curve was previously used by James and Shih (1973) on two different pathosystems (McRoberts et al., 2003). Limitations associated with practical use of incidence-severity relationships are essentially derived from their inconsistency in relations to location, season, stage of epidemic, crop management systems and host genotype variations (Cardoso et al., 2004). Once the model has proven robust across all these, one may opt to use the easily measured parameter (incidence) (Sweetmore et al., 1994; Silva-Acuna et al., 1999; Cardoso et al., 2004). Therefore we recommend the use of leaf incidence in place of severity in genotype and fungicide screening trials, 173 describing models for economic thresholds or epidemics studies of cashew leaf anthracnose. However, caution is needed since the cashew leaf anthracnose severity or incidence link to the panicle or nut anthracnose incidence/severity has not been established. We observed that the prevailing climatic (rainfall) conditions at flowering or fruiting stage play a major role in predicting leaf anthracnose before nut setting. This is in conformity with previous finding in Brazil where severity of anthracnose was coupled with rainfall and flushing of cashew (Cardoso et al., 2000).

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

In our model, we considered severity as independent variable and incidence as the dependent: Anthracnose is a polycyclic disease (Agrios, 2005). Changes in incidence over time are

determined by the dynamics of severity or sources of inoculum at initial stages of epidemics (McRoberts et al., 2003). By exploring the regression curve minimum and maximum limits derived from the incidence-severity relationship, we have assessed the propensity of the environment for the disease epidemics across different sites, crop seasons, genotype combinations and production system. Anthracnose spread was clearly associated with rainfall during the first week of July. In general this coincided with the flushing peak for most clones involved in the trials. This in agreement with knowledge that dispersion of anthracnose inoculum is by rain splashes (Ponte, 1984; Intini, 1987; Freire and Cardoso, 2003).

When the relationships between pairs of incidence and severity are mathematically expressed and consistent at multiple locations or environments, data from individual sites can be pooled into a summary equation 198 without prejudice to proper interpretation (Cardoso et al., 2004). In this study overall means for essential coefficients such as eii and emi, were used to generate the summary equation that explained the relationships between anthracnose incidence and severity across different environments.

When incidence data of 1, 5, 10, 40 and 60% were computed in to the inverse equation (2), severity estimates were returned as 0.01, 0.05, 0.10, 0.50 and 1% respectively.

$$S= (-1/a)Ln(1-(1/b)*I) \quad (2)$$

Where *S is* for severity; *a* for estimated initial disease incidence (eii); *b* for estimated maximum incidence (emi), Ln for natural logarithm and I for incidence,

This indicates that very low levels of severity are associated with increased alloinfection.

In this model, both incidence and severity were found to increase. When incidence approached a maximum of 80%, the severity was only around 5%. Then, only severity continued to increase up to a maximum of 25%. This pattern of post-maximum incidence increase of severity has been discussed by Cardoso et al., (2004). The spread of the disease may be limited because severely

infected senescent leaves tend to drop off and the un-infected ones (20%) may be reaching maturity and therefore inhibiting fungal penetration.

In this study we adopted the scale developed by Nathaniels, (1996) initially used for cashew powdery mildew (*Oidium anacardii* Noack). In previous studies, cashew leaf anthracnose was assessed based on whole canopy scores (Anon, 1999; Cardoso et al.,1999; Cardoso et al., 2000; Cardoso et al., 2002), without standardized pictorial diagrams thus making it difficult to use by other workers.

An adult common type cashew tree grows extensively towards the end of the rainseason and reproductively when the temperature declines (Wunnachit and Sedgley, 1992). Dwarfs and young trees, tend to grow continuously (Milheiro and Evaristo, 1994). Thus when the environment 223 is favorable, two peaks of the disease epidemic may be observed in a year (Cardoso et al., 2000). Our method which is young leaf based has the advantage of being able to separate the two epidemics accurately. This method can be applied in all other tree crops with two flushes per year.

Estimation of cashew anthracnose damage through its incidence on young leaves has proven to be a more effective, faster, more accurate and user friendly method than severity scores. This is in line with Sweetmore et al., (1994) who found incidence data to be simpler to collect and less subjective than severity and thus recommend for larger scale surveys. However, special attention may be necessary when assessing cashew anthracnose where other similar but distinguishable leaf diseases such as leaf blight (Sijaona et al., 2005) and Pestalotiopsis (Intini and Sijaona, 1983) are present.

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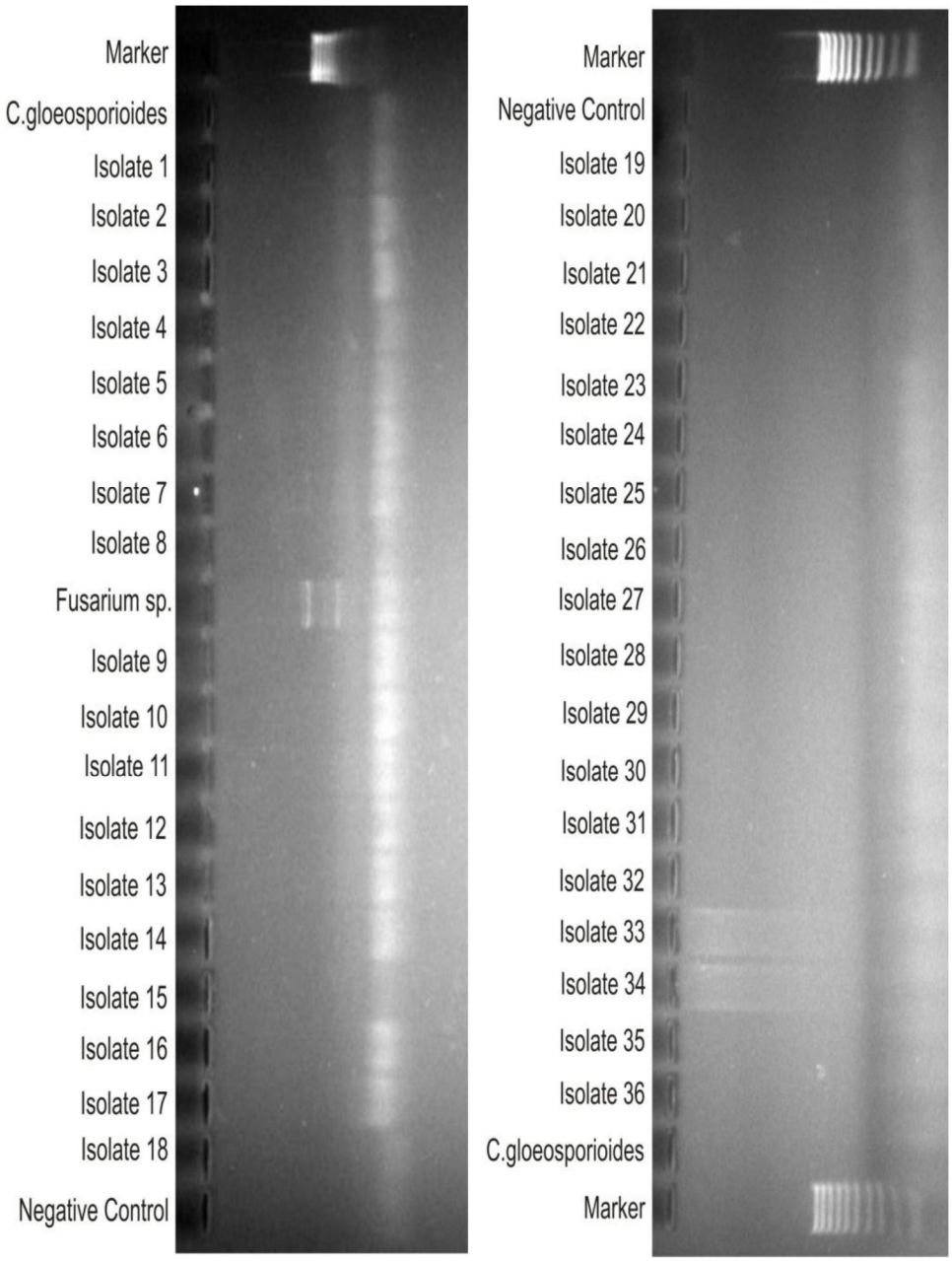


Fig. 1

Taxon-specific amplification products of genomic DNA from *Colletotrichum gloeosporioides* isolates collected from different parts of Mozambique. Negative control lane contained all reagents except DNA. *C. gloeosporioides* from mango, was used as positive control. DNA from *Fusarium* sp. isolated from cashew was also included for contrasting results.

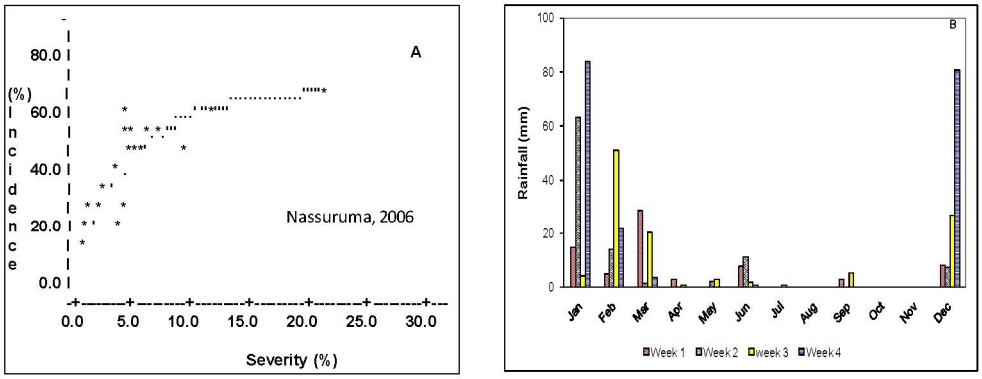


Fig. 2a

Cashew anthracnose severity and incidence relationships on dwarf genotypes (A) and rainfall distribution (B) at Nassuruma, Mozambique. Vertical bars represent rainfall means per week.

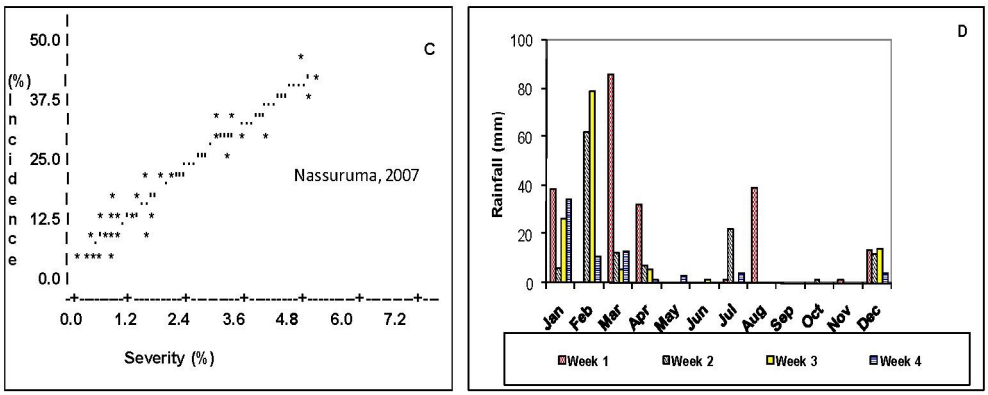


Fig. 2b

Cashew anthracnose severity and incidence relationships on dwarf genotypes (C) and rainfall distribution (D) at Nassuruma, Mozambique. Vertical bars represent rainfall means per week.

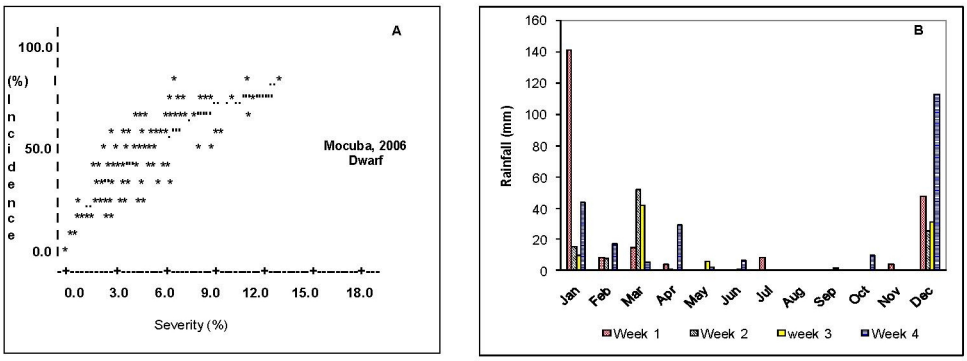


Fig.3a

Cashew anthracnose severity and incidence relationships on dwarf genotypes (A) and rainfall distribution (B) at Mocuba, Mozambique. Vertical bars represent rainfall means per week.

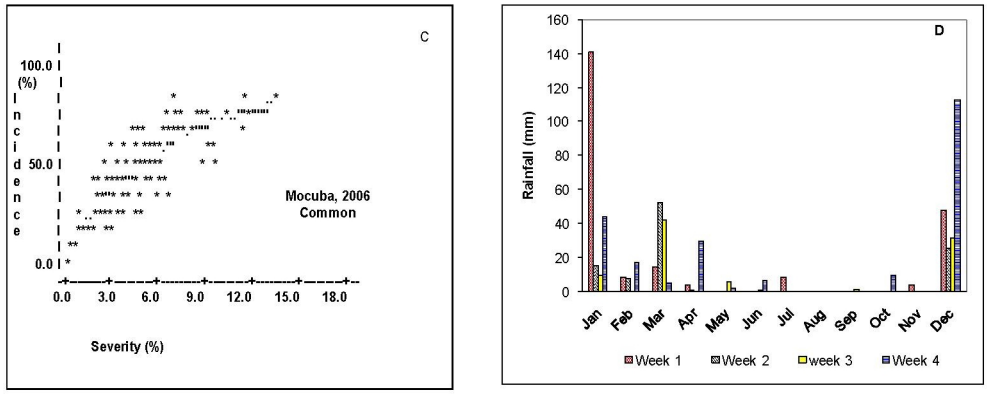
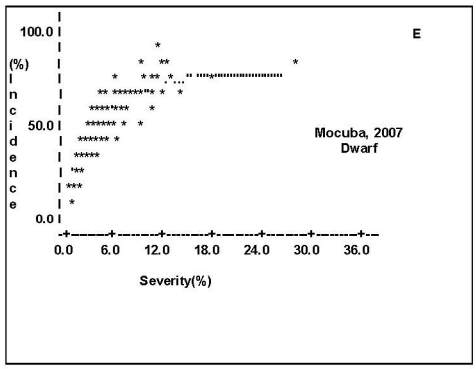


Fig.3b

Cashew anthracnose severity and incidence relationships on Common genotypes (C) and rainfall distribution (D) at Mocuba, Mozambique. Vertical bars represent rainfall means per week.



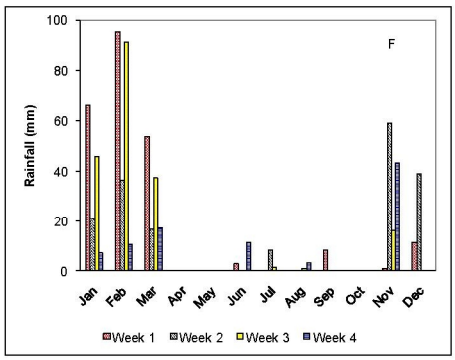
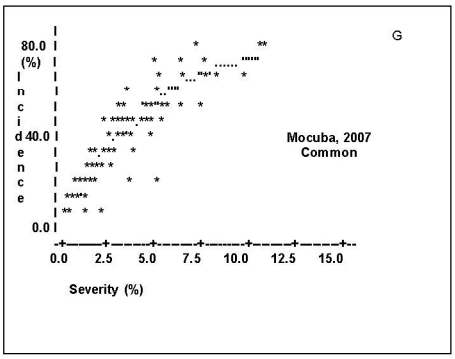


Fig.3c

Cashew anthracnose severity and incidence relationships on dwarf genotypes (E) and rainfall distribution (F) at Mocuba, Mozambique. Vertical bars represent rainfall means per week.



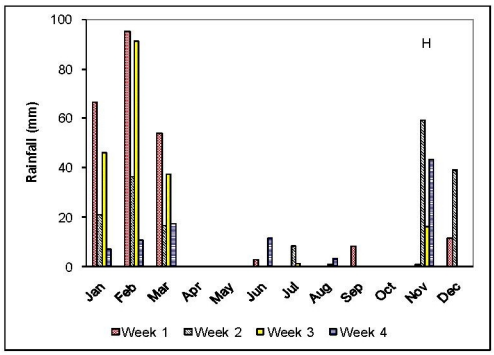


Fig.3d.

Cashew anthracnose severity and incidence relationships on dwarf genotypes (G) and rainfall distribution (H) at Mocuba, Mozambique. Vertical bars represent rainfall means per week.

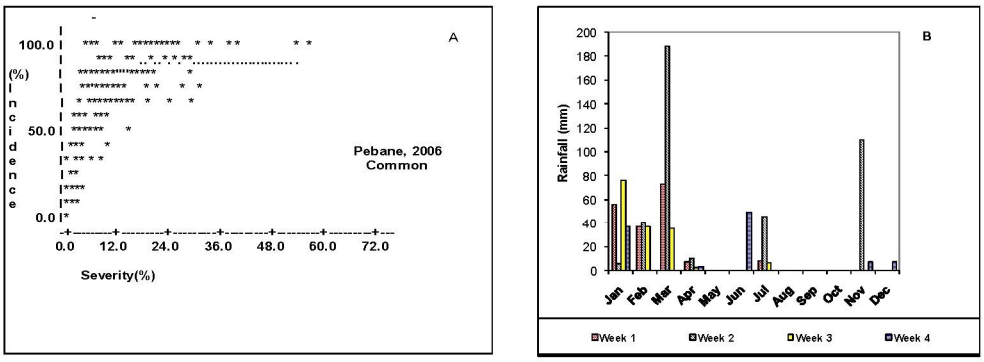


Fig.4

Cashew anthracnose severity and incidence relationships on common genotypes (A) and rainfall distribution (B) at Pebane, Mozambique. Vertical bars represent rainfall means per week.

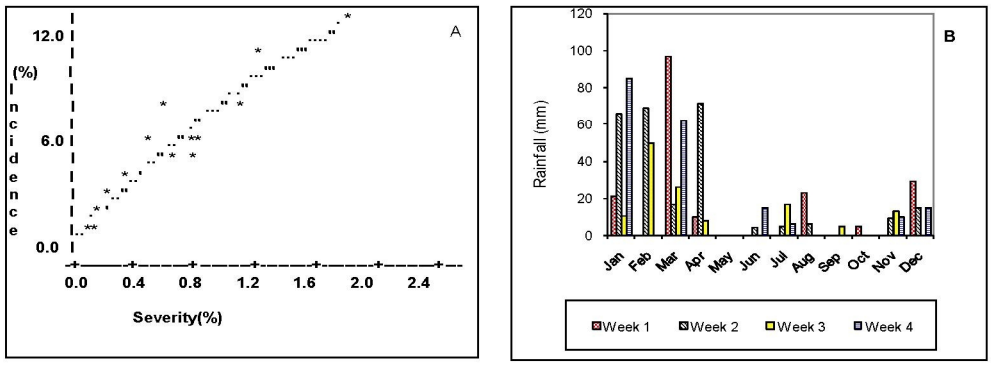


Fig.5

Cashew anthracnose severity and incidence relationships on mixed genotypes treated with triadimenol (A) and rainfall distribution (B) at Rapale, Mozambique. Vertical bars represent rainfall means per week.