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Full Length Research Paper

A canker and dieback disease threatening the cultivation of *Grevillea robusta* on small-scale farms in Kenya

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A canker and dieback disease was recently reported on *Grevillea robusta* in east Africa but little was known about its magnitude, distribution and associated pathogens. In our survey of the disease approximately 36% of 17,994 *G. robusta* trees assessed showed canker and dieback symptoms. Disease index increased from the humid zone (18%) and to the semi-arid zones (67%). Tree mortality increased from 0.3 to 20% respectively on the same scale and was highest in trees less than 7 years old in all zones. Disease index and tree mortality showed positive correlations with drought period which increased from an average of 0.7 months (humid) to 7 months (semi-arid zones). Both variables showed negative correlations with farm altitude. Disease index was higher in the woodlots compared to other types of tree planting in all zones. Two *Botryosphaeriaceae* species, *Neofusicoccum parvum* and *Lasiodiplodia theobromae* known to be pathogenic on *G. robusta* were highly abundant in severely diseased stems and branches and their occurrence also showed positive correlations with disease severity. From the results of this study, it was recommended that *G. robusta* should not be planted in the semi arid zones of Kenya, due to its susceptibility to the canker and dieback disease.

Key words: Agro-ecological zones, agroforestry, Botryosphaeriaceae, canker and dieback disease, disease index, *Grevillea robusta.*

INTRODUCTION

Grevillea robusta Cunn ex R. Br. is a fast growing multipurpose tree that provides various goods and services that include construction material, fuel wood, shade, fodder and soil fertility improvement (Muchiri et al., 2002). *G. robusta* was introduced into Kenya as a shade and windbreak tree for tea plantations more than 100 years ago (Harwood, 1989). The tree has been adopted as a major agroforestry tree species and has become a major timber species on small-scale farms, where it is significantly contributing to household income (Holding et al., 2006). Due to its ability to tolerate heavy pollarding and pruning of branches, the farmers find it suitable for agroforestry, a farming system that includes planting trees together with agricultural crops. Tyndall(1996) reported that, about 96% of small scale farmers grew *G. robusta* trees in the humid to the semi humid areas in Central Kenya highlands.

In Kenya, canker and dieback symptoms were first reported on *G. robusta* in 1960 (Smith, 1960) and later in the 1980s (Milimo, 1988). No isolations of pathogens were done and the species continued to be regarded as having “no disease or pest of economic importance worldwide” (FAO, 2001). However, reports of canker and dieback symptoms in semi-arid areas together with results from monitoring of on-farm experiments showed that, the incidence of canker and dieback symptoms in *G. robusta* increased from 18 to 67% and mortality of the trees also increased from 2 to 18% between 2001 and 2003 (Njuguna, 2003). Diseased trees showed poor growth compared to healthy ones. On diseased trees, cracks on stems or branches resulted in rupturing of the

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Table 1. Characteristics of the agro-ecological zones included in the study.

Characteristics	Agro-ecological zone (AEZ)*)				
	Humid (AEZ1)	**Sub-humid (AEZ2)**	**Semi-humid (AEZ3)**	**Semi-humid/semi-arid (AEZ4)**	**Semi-arid (AEZ5)**
Altitude (m) above sea level (a.s.l)	>2000	1500-2000	1000-1500	700-1000	400-700
Annual rainfall (mm) bimodal	1200-1400	1100-1200	900-1100	500-900	<500
Mean annual temperature (℃)	13-15	5-18	18-20	21-24	23-24
Mean min temperature (℃)	Seasonal frost	9	12.5	14	16
Mean max temperature (℃)	18	22	25	32	34
Production potential	High	High–moderate	Moderate	Low-marginal	Marginal

*Agro-ecological zone characteristics were adapted from Jaetzold and Schmidt (1983).

bark followed by resin exudation. As cankers increased, girdling of young stems, branches and shoots led to dieback of shoots, branches and death of trees. Similar symptoms were reported on the species in Uganda where *Neofusicoccum parvum* (Pennycook and Samuels) Crous, Slilppers and Phillips [*Botryosphaeria parva* Pennycook and Samuels] was shown to be pathogenic on *G. robusta* seedlings (Toljander et al., 2007). In a related mycological study two species, *N. parvum* and *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl., were found to be the most frequent species associated with the canker and dieback symptoms on *G. robusta* in Kenya. (Njuguna et al., Swedish University of Agricultural Sciences, unpublished data). Pathogenicity tests in glasshouse experiments further showed that *N. parvum* and *L. theobromae* were highly pathogenic on young seedlings of *G. robusta* (Njuguna et al., Swedish University of Agricultural Sciences, unpublished data). *Neofusicoccum. parvum* and *L. theobromae* are serious canker and dieback pathogens on many herbaceous and woody plants worldwide (Úrbez et al., 2008, Slippers and Wingfield, 2007; Agrios, 2005; Sinclair and Lyon, 2005). Although members of the genus Proteaceae, to which *G. robusta* belongs, were reported to be susceptible to a variety of Botryosphaeriaceae pathogens in South Africa (Marincowitz et al., 2008; Denman et al., 2003), information on the magnitude and distribution of Botryosphaeria canker and dieback disease on *G. robusta* in East Africa is scarce.

The main aim of the study was to assess the magnitude and distribution of the canker and dieback disease in *G. robusta* based agroforestry systems in five agro-ecological zones in Kenya. The specific objectives were to:

(1) Assess the variations in disease incidence and severity of the canker and dieback disease on *G. robusta* on small scale farms in five agro-ecological zones;

(2) Assess the disease variables on different tree planting types (niches) and tree ages and; (3) Determine the relationships between the disease variables, farm altitude and drought in each agro-ecological zone and;

(4) Assess the occurrence of the pathogens on various plant parts. By the time of this study, no systematic study had been done to isolate the causes or to determine the magnitude of the disease in the country.

MATERIALS AND METHODS

Study area and agro-ecological zones

The study was conducted in 2005 in Kenya in three districts that stretched from the slopes around Mount Kenya with both humid and semi-humid agro-ecological zones (AEZs) in Kirinyaga district, the semi-humid zones in Maragua district to the semi-arid lowland zones in Kitui district. Kirinyaga district is located between latitude 0° 1' and 0° 40' south and longitude 37° and 38° east covering an area of 1,478 km^2 at altitudes 1100 m to over 6000 m above sea level (a.s.l.). Maragua district is located between latitude 0° 45' south and 1° 7' south and longitude 36° east and 37° 59' east covering an area of 868 km^2 at altitudes between 1100 m and 2950 m a.s.l. Kitui district is located between latitude 1° 21' and 3° 5' south and longitude 37° and 39° east covering an area of 20,462 km^2 at altitudes between 400 and 1600 m a.s.l. The agro-ecologicalzones used in this study were defined by annual rainfall and temperature (Table 1). Kenya experiences a bimodal type of rainfall with two rainfall periods; the long (730 mm) rains alleys or terraces, (3) trees scattered in cropland and (4) wood lots. The main type of planting *G. robusta* was recorded for each farm. In addition, the ages of the trees sampled were recorded and classified into five age-classes; (1) 1 to 5 years, (2) 6 to 10 years, (3) 11 to 15 years, (4) 16 to 20 years and (5) >21 years.

Table 2. Farm size, tree numbers, tree age and disease index on canker and dieback disease on *G. robusta* in five agro-ecological zones in Kenya. Data was pooled for all farms in each zone.

	Agro-ecological zone				
	Humid (AEZ1)	**Sub-humid (AEZ2)**	**Semi-humid (AEZ3)**	**Semi-humid-semi arid (AEZ4)**	**Semi-arid (AEZ5)**
Number of farms sampled	18	21	24	17	15
Average farm size (ha) ±SE	2.9±0.3 a	2.5±0.3 a	2. 7±0.3a	2.8±0.3a	2.9±0.2a
Total number of trees assessed	3309	5556	6027	1861	1241
Mean number of trees per farm*±SE	184±13a	265±33b	251±46b	110±9 c	83c±7c
Median tree age in years**	7 (1-30)	8 (1-35)	8 (1-39)	7 (1-22)	5 (1-15)
Mean disease index *±SE (P<0.001, adjusted R2= 68.9)	17.6±1.2a	18.6±2.1a	29.1±2.7b	49.1±3.8c	67.1±3.9d

*Means followed by different superscript letters were significantly different. ** Figures in brackets show the age of youngest and oldest tree in each agro-ecological zone.

Disease incidence and severity assessment

translation elongation factor 1-alpha (TEF 1-alpha) gene region. The methods of extracting, amplification and sequencing of the ITS region and the translation elongation factor 1-alpha were as described by Slippers et al. (2004). The occurrence of the emerging canker forming fungi on the different plant parts was recorded.

Statistical analysis

Data collected were analyzed using Minitab Version 15 (Minitab Inc 2009). Summary statistics were used to calculate the total and mean number of trees occurring in each AEZ, type of planting and also tree ages. Scatter plots showed that disease incidence, severity and tree mortality data were not normally distributed.

Since logarithmic transformation produced the best fit, the data on these variables were log10(x+1) transformed before analysis. Analysis of variance (ANOVA) was used to test the significance of AEZs, type of tree planting and farm altitude on disease incidence, severity and tree mortality on *G. robusta*. Pearson's correlation was used to show the relationships between disease incidence, severity and tree mortality and also between abundance of canker fungi and disease severity. The relationships between disease incidence, severity and mortality with farm altitude and number of continuously dry months (drought) were determined using simple linear regression. One-way ANOVA was used to test the differences in occurrence of potential canker pathogens on different parts of *G. robusta*. In all tests data were pooled for all farms in each agro-ecological zone during analysis.

RESULTS

Distribution of *G. robusta* trees in agro-ecological zones and farms

In the survey 17,994 trees were assessed in 95 farms, whose average size ranged between 2 to 3 ha in the five agro-ecological zones (AEZs). The mean number of trees per farm differed between zones from 83 in the semi-arid zones to 265 in the semi-humid zone ($P < 0.001$) (Table 2). As indicated by the standard deviations there was great variability in the number of trees on farms in the sub-humid and semi-humid areas compared with the other zones. The percentage of trees encountered in each age-class was 32% in 1 to 5 years, 41% in 6 to 10 years, 17% in 11 to 15 years and 10% in those over 16 years. The oldest tree sampled was 39 years in the semi-humid zone (Table 2).

About 55% of the trees were planted on boundaries, 17% on woodlots, 15% on terraces and alleys and 13% were scattered among crops (Table 3). In the humid, sub- humid and semi-humid zones, boundary planting was the most common type of planting comprising 66% of the trees, which were usually planted at very close spacing, <2 m apart. In the semi-humid /semi-arid and semi-arid zones, woodlots were popular comprising 47% of the trees planted. Planting along terraces, alleys and trees scattered on cropland did not differ between zones.

Field disease symptoms observed

The disease was characterized by dieback of young shoots and branches leaving naked shoots and branch tips which were the main symptoms in the humid and sub-humid zones (Figure 1A). In the semi-humid to the semi-arid areas, dieback was severe (Figure 1B), accompanied by cracks, branch and stem cankers without resin (Figure 1C) or serious stem cankers with resin (gummosis). Resin was initially creamish turning yellow and dark brown with time (Figure 1D). Cankers varied in size from small lesions of few millimeters to large open wounds sometimes

Table 3. Percentage (means ± SE) disease index and mortality of *G. robusta* trees in different types of tree planting on-farm in five agro-ecological zones in Kenya: Disease index and tree mortality data were pooled for all zones.

Type of planting	Trees assessed	Disease index	Mortality
		Mean ± SE	
Boundaries	9897	27.0 ± 2.6a*	3.3 ± 0.1a
Terraces and alleys	2699	35.4 ± 4.4b	5.9 ± 0.4b
Mixed with crops	2339	40.0 ± 6.8b.	7.1 ± 0.3c
Woodlots	3059	50.3 ± 6.1c	13.2± 0.4d

*Means followed by the same superscript letter are not significant within columns at the 95% confidence level.

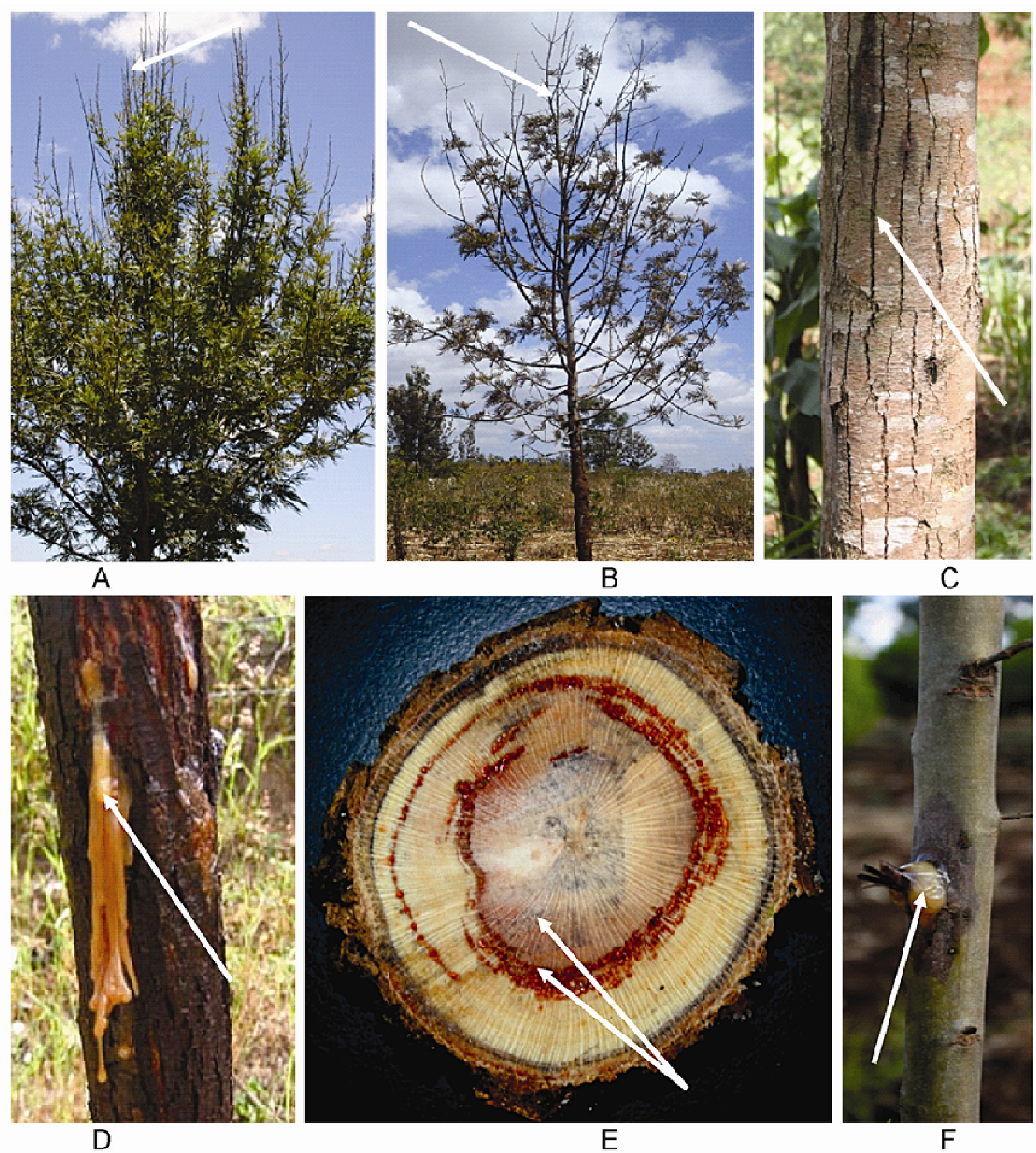

Figure 1. Symptoms of stem cankers and dieback on *G. robusta* in Kenya: (A) Shoot and branch dieback only, (B) Severe dieback on shoots and branches, (C) Stem cracks and small cankers, (D) Resin ooze from stem (trunk) cankers, (E) Damaged growth rings; resin ooze left arrow and; and infected heartwood, right arrow (F) Infection spread from infected tree to healthy tree through pruning of branches.

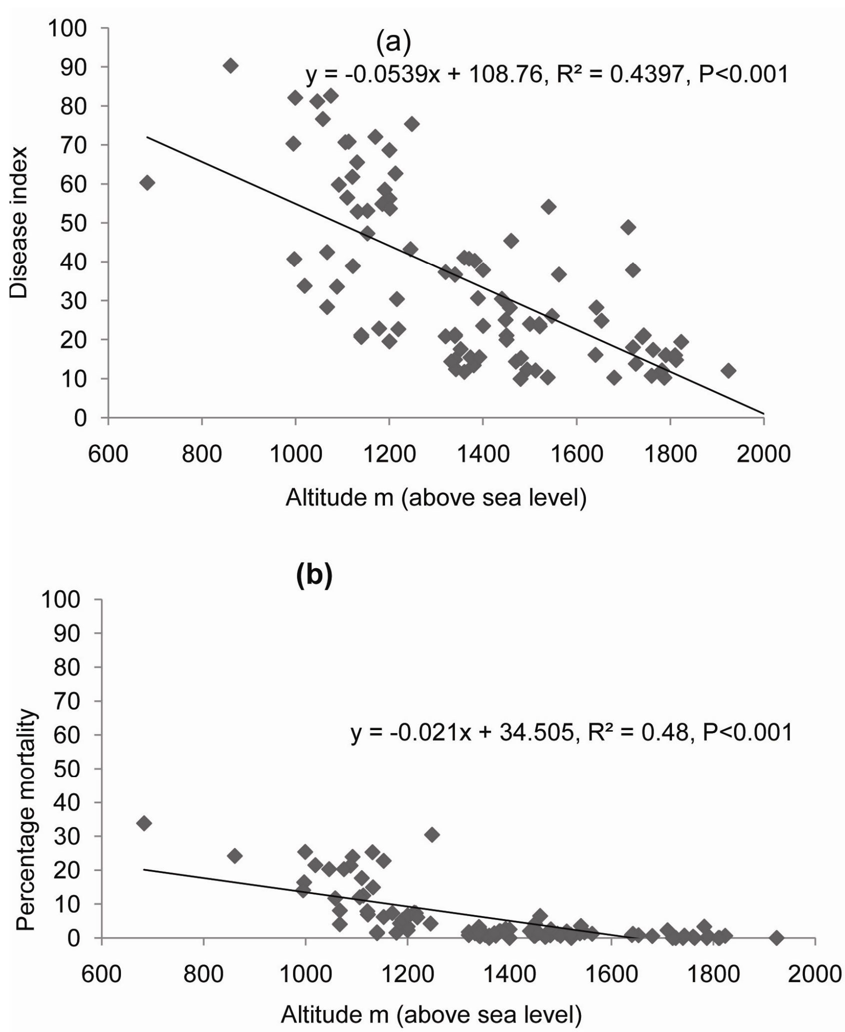

Figure 2. Relationships between canker and dieback disease and altitude, (a) disease indices (calculated from diseases incidence and severity values) and (b) mortality of *Grevillea robusta* trees.

extending over 1m along the stem on severely infected trees (dying or dead trees).

Relationships between farm altitude and drought with disease variables on *G. robusta*

Farm altitude showed significant negative relationships with disease index (Figure 2a) and mortality (Figure 2b). There was a clear increase in disease index and tree mortality below 1200 m a. s. l. The numbers of resinous stem cankers per tree and amount of resin produced seemed to increase with drop in altitude from the higher to the lower altitudes where termite damage was also prevalent. The length of drought periods also showed positive correlations with disease index ($P<0.001$, Pearson corr. = 0.89). In addition supportive rainfall data from the Kenya Meteorological Department for the period 1990 to 2005 also showed that, the number of *parvum* and *L. theobromae* were also isolated from pruning wounds (Figure 1F) and from both healthy and infected flower buds. About 75.5% of the trees with canker and dieback symptoms also showed higher presence *N. parvum* on canker and dieback symptoms at 38.1% compared to *L. theobromae* at 33.2%. Further, these two fungal species showed positive correlations ($P<0.001$, Pearson corr. = 0.717) with canker and dieback disease severity.

DISCUSSION

The present study showed that 35.6% of the trees were diseased and that this magnitude verified the existence of serious canker and dieback disease on G. robusta in Kenya. Since the same disease symptoms (Figure 1)

Table 4. Occurrence of seven *Botryosphaeria* species on healthy and diseased plant parts shoots, branches and stems of *G. robusta* trees in Kenya.

Accession number	Fungus species	Healthy			Diseased			
		Shoots	Branches	Stems	Shoots	Branches	Stems	P-Value
		Mean isolation frequency* ±SE						
FJ904893	*Neofusicoccum parvum* (Pennycook and Samuels) Crous, Slippers and Phillips	2.6±0.67a	1.8±0.18b	2.5±0.21a	5.2±0.3c	5.1±0.2c	5.6±0.1d	0.001
FJ904889	*Lasiodiplodia theobromae* (Pat.) Griffon and Maubl.	2.7±0.25a	1.9±0.18b	2.4±0.13a	4.9±0.3a	4.8±0.3b	5.6±0.2b	<0.001
FJ904868	*Diplodia seriata* De Not.	2.0±0.0a	2.2±0.13a	2.1±0.14a	4.3±0.3b	3.7±0.3c	4.7±0.2d	0.012
FJ904864	*Botryosphaeria* sp. Ces. and De Not.	-	-	-	3.4±0.8b	2.7±0.5b	3.6±0.2b	0.416
FJ904901	*Diplodia* sp. Fr.	-	-	-	3.3±0.7a	3.7±0.4a	3.7±0.4a	0.891
FJ904833	*Lasiodiplodia pseudotheobromae* Phillips, Alves and Crous	-	-	2.0±0.0a	1.8±0.4a	2.7±0.3a	2.2±0.2a	0.28
FJ904884	*Lasiodiplodia parva* Phillips, Alves and Crous	-	-	-	2.3±0.1a	2.4±0.2a	2.4±0.2a	0.143

*Mean number of isolations per sample out of 12 isolation attempts. N = 3,305 samples (2,850 diseased and 441 healthy samples). Means followed by the same superscript letter are not significantly different within rows at the 95% confidence level.

were observed in Uganda (Toljander et al., 2007), the disease could be considered widespread. Further, the Botryosphaeriaceae fungi *N. parvum* and *L. theobromae* were closely associated with the canker and dieback disease in both countries (Table 4) and Toljander et al. (2007). The variation in the number of *G. robusta* trees planted in the different agro-ecological zones seemed to reflect influence of farming systems and farm sizes in determining the number of trees planted and also type of tree planting in each zone.

In the humid and sub-humid zones, the conditions favored growth of *G. robusta* (Booth and Jovanovic, 2002; Table1), but these two zones were also important for tea and coffee growing thereby limiting tree planting to farm boundaries. These cash crops do not grow well in the semi-humid zone, where most *G. robusta* trees were found. In this zone, trees have become an important alternative source of income through sale of timber and fuel wood (Holding et al., 2006). Occurrence of high numbers of less than 22 years old trees in the semi-humid/semi-arid and semi-arid zones suggested that, tree planting was a recent practice in these zones. In addition, during the monitoring studies, we observed that mostyoung trees died before establishing, especially inthe semi arid areas explaining why there were fewer trees of less than 5 years compared to those between seven and ten years. In these zones, agroforestry technologies that include tree planting were being promoted as an effort to rehabilitate the semi-arid and arid lands (Kigomo, 2003; Scherr, 1992) and to cushion farmers from the frequent crop failure as a result of prolonged droughts. The presence of 1-year-old trees showed that, tree planting was still active in all zones

The variation in disease index and mortality observed on *G. robusta* trees between the farms and also zones (Figure 3) mirrored the variations in the prevailing biophysical characteristics. These variations could be explained by differences brought by prevailing soil type, general location of each farm whether it was valley bottom, flat ground or slope of each farm (Njuguna et al., Swedish University of Agricultural Sciences, unpublished data), which were directly related to farm altitude. Altitude which was also connected to the amount of annual rainfall received in the agro-ecological zones was an important factor in influencing disease magnitude. The high negative correlations between altitude, disease index and tree mortality showed that the lowlands were not suitable for growing *G. robusta* trees. The occurrence of many months of drought in these areas seemed to favor the development of the disease on *G. robusta*. These results contradict the suggestions made by Raju (1992) that *G. robusta* could be promoted for planting in the semi-arid and arid areas.

The Botryosphaeriaceae are associated with a wide variety of plants in the tropics and temperate areas (Slippers and Wingfield 2007) existing mainly as saprophytes, endophytes and latent or opportunistic pathogens. They infect their hosts through natural openings such as stomata, lenticels and also through wounds (Smith 1995).

Many authors have shown that diseases caused

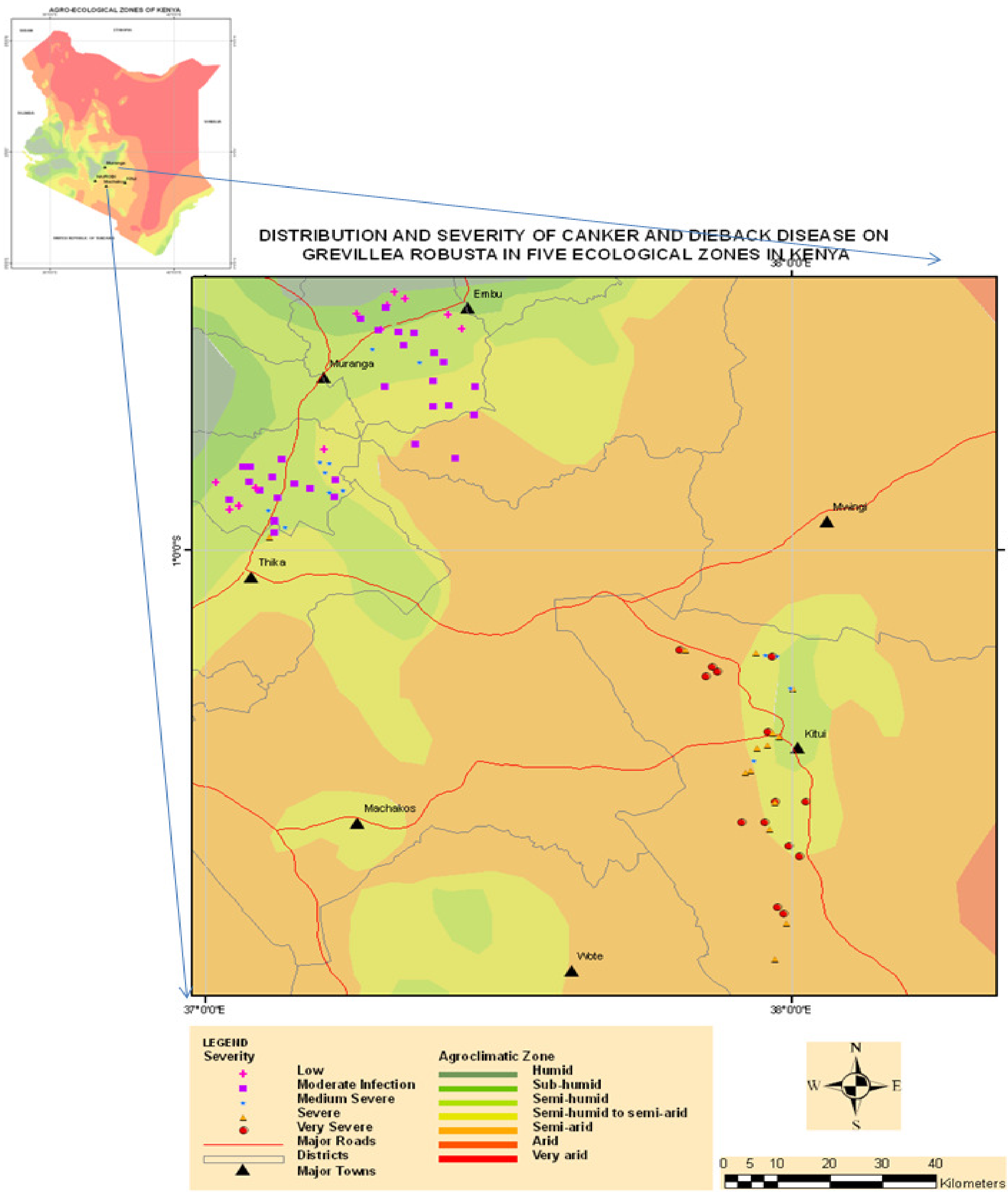

Figure 3. Supplementary material: Map of farm distribution within the agro-ecological zones.

by the Botryosphaeriaceae fungi are usually linked to environmental stress factors acting on the host species (Úrbez-Torres and Gubler, 2009; Pavlic et al., 2007). Desprez-Loustau et al. (2006) and Shoeneweiss (1981) also showed that environmental stresses such as drought predisposed plants to infection by endophytes or opportunistic pathogens; indicating that host stress might trigger a shift from the latent to the pathogenic phase of some endophytic fungi including the Botryosphaeriaceae. In this study, it was possible that the prolonged drought periods could have predisposed *G. robusta* to infection by the *Botryosphaeria* pathogens especially in the semi-arid areas. The occurrence of *N. parvum* and *L. theobromae* seemed to coincide with high levels of disease index in the semi arid areas.

The disease seemed to develop from actively growing tissues in young shoots, and inflorescences, and progressed into the branches and stems. In most cases girdling of both shoots and branches resulted in death of shoots and branch tips. Disease progression from young infected shoot tissues (that is the most recent growth ring) could also develop further into the stem and to older growth rings and in this way could have provided links between infected young tissues and stem cankers. The cankers occurred as swollen stems with cracks through which resin oozed out, probably as a result from pressure from infected collapsed tissues inside the stem (Figure 1D). Internally, fungal infection could spread between growth rings through weakened tissues (Figure 1E). Dieback of shoots and branches and gummosis leading to dark bark and destruction of growth rings was characteristic of Botryosphaeria disease symptoms and showed similarity to Botryosphaeria symptoms described on *Eucalyptus* spp. (Roux et al., 2001; Gezahgne et al., 2004; Slippers and Wingfield, 2007). The high isolation frequency of *N. parvum* and *L. theobromae* on severely diseased stems and branches and the positive correlations between the isolation frequency of these two fungal species and disease severity indicated that, they could be the main causes of the canker and dieback disease (Table 4). Pathogenicity tests further showed that *N. parvum* and *L. theobromae* were highly pathogenic on young seedlings of *G. robusta* (Njuguna et al., Swedish University of Agricultural Sciences, unpublished data). while *D. seriata* was only weakly pathogenic in young *G. robusta* seedlings. Many authors have further shown that *N. parvum* [*B. parva*] and *L. theobromae* [*Botryosphaeria rhodina*] are highly pathogenic on many plant hosts including members of the Proteaceae to which *G. robusta* belongs (Toljander et al., 2007; Denman et al., 2003; Roux et al., 2001). Our pathogenicity results further showed that *N. parvum* and *L. theobromae* were highly pathogenic while *D. seriata* was moderately or weakly pathogenic on *G. robusta* seedlings (Njuguna et al., Swedish University of Agricultural Sciences, unpublished data). The disease seems to involve a number of species in the Botryosphaeriaceae as shown by other authors (Pavlic et al., 2007).

The large coverage of *G. robusta* may have facilitated spread of the disease over large areas. High disease incidence and mortality in woodlots, where the trees were closely spaced, compared to other types of planting possibly provided easier disease spread between trees. Concurrently competition for water might have increased stress among the trees. Pruning of trees seemed to aid disease spread within farms from infected plants to healthy plants through pruning tools. Fresh cuts or open wounds provide sources of direct entry of many fungi into the host system (Davison and Tay, 2008; Slippers and Wingfield, 2007; Rumbos, 1997). Infection on flowers also indicated the possibility of the disease to be seed borne, as has been proved in other tree species, that are also hosts to the Botryosphaeriaceae fungi (Gure et al., 2005; Cilliers et al., 1995). The emergence of a disease with such a wide plant host range will not only threaten *G. robusta* but also other agroforestry trees. Currently, the disease is a clear threat to many on-farm trees that include species of Eucalyptus, *Mangifera indica* L., and *Syzygium cordatum* Hochst. ex Krauss and other Proteaceae (Rodas et al., 2008; Slippers et al., 2005; Gezahgne et al., 2004; Marincowitz et al., 2008; Pavlic et al., 2007; Burgess and Wingfield, 2001) within the eastern and south African region. Results from this survey in which approximately 36% of the trees were diseased clearly showed that the disease was widespread and more studies are needed to understand its implication in agroforestry systems in Kenya. In addition, the presence of the disease on *G. robusta* in Uganda (Toljander et al., 2007) is an indication that, the disease is serious within the East African region. These results further showed that, the species can no longer be considered as “healthy”. The study recommends that, due to the high susceptibility to the disease in the semi-arid areas, *G. robusta* should not be planted in areas with long periods of drought.

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