EFFECTS OF NARROWING GENETIC BASE AND ABIOTIC STRESS ON LEAF SPOTTING IN *GREVILLEA ROBUSTA*

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KIMATU JN. 2011. Effects of narrowing genetic base and abiotic stress on leaf spotting in *Grevillea robusta*. *Grevillea robusta* is a widely grown agroforestry tree and is regarded as a pioneering coloniser of disturbed sites. Our current understanding on changes of species due to disturbance, abiotic conditions and biotic interactions is very minimal. We investigated a leaf-spotting disease and abnormal growths on *G. robusta* in Yala and Kodera forest plots in Nyanza province, Kenya. The study comprised symptomology, identification of causal pathogens as well as tissue and soil analyses. *Phyllosticta* spp. and *Pestalotia* spp. fungi were isolated from the leaf spots. Seedling reinoculation confirmed *Phyllosticta* spp. as the lesser opportunistic cause of the leaf spot. However, mineral and proteoid root analyses suggested that abiotic and genetic factors were the main causes of the leaf spotting. The Yala forest had lower pH, phosphorus toxicity (> 0.07%) and poor water drainage, while the Kodera forest had generally high manganese toxicities in soil and leaf tissue.

Keywords: Agroforestry, toxicity, leaf spots, proteiod roots

KIMATU JN. 2011. Kesan pangkalan genetik yang semakin mengecil dan tekanan abiotik terhadap perbintikan daun dalam *Grevillea robusta*. *Grevillea robusta* merupakan pokok hutan tani yang ditanam secara meluas dan dianggap sebagai penjajah perintis di tapak terganggu. Pemahaman semasa kita tentang perubahan spesies akibat gangguan, keadaan abiotik dan interaksi biotik sangat kurang. Kami mengkaji penyakit perbintikan daun dan pertumbuhan yang luar biasa pada pokok *G. robusta* di plot hutan Yala dan Kodera di wilayah Nyanza, Kenya. Kajian meliputi pemerhatian gejala, pengecaman patogen penyebab serta analisis tisu dan analisis tanah. Kulat *Phyllosticta* spp. dan *Pestalotia* spp. diasingkan daripada bintik daun. Penginokulatan semula anak benih mengesahkan yang *Phyllosticta* spp. kurang mengakibatkan perbintikan daun. Namun analisis mineral dan analisis akar proteoid mencadangkan yang faktor-faktor abiotik dan genetik merupakan penyebab utama perbintikan daun. Hutan Yala mempunyai pH dan ketoksikan fosforus (> 0.07%) yang lebih rendah serta saliran air yang lemah manakala hutan Kodera secara amnya mempunyai ketoksikan mangan yang tinggi di dalam tanah dan tisu daun.

INTRODUCTION

Grevillea robusta is an agroforestry tree native to subtropical eastern Australia and has extensively been adopted into agricultural farming systems of Sri Lanka, India, Rwanda, Burundi, Kenya, Uganda, Tanzania and Zimbabwe (Midgley 1983). The initial introductions of *G. robusta* seed to some countries were most probably made from a narrow genetic base of a few trees. In most countries, the origin of the initial introductions is unknown. Studies of 20 isoenzyme loci assays using 23 natural populations of *G. robusta* in Australia showed that heterozygosity per population (He) was between 0.080 and 0.131. However, the genetic diversity of individual populations was not related to ecological characteristics (Harwood et al. 1997). Records of seed transactions by CSIRO's Australian Tree Seed Centre indicate that very little seed has been supplied from Australia to other countries in the last few decades; most of that which has been supplied has been collected from street trees of unknown provenance. Hence, there is a likelihood of inbreeding which can lead to substantial reduction in vigour and reduced adaptability. Isozyme studies indicate that genetic diversity is lower in the African land races than in the natural population (Harwood 1989). Habitat fragmentation and continued propagation of *G. robusta* due to its agroforestry demand can cause homogenisation and rapid genetic

differentiation which can lead to inbreeding and loss in genetic diversity (Zhang et al. 2009). Habitat fragmentation can expose populations to stochastic process (Lande 1988) and increase a species genetic decline and eventual extinction possibility (Ford et al. 2001), especially if there is a decline in genetic fitness (Lande 1993).

Grevillea robusta leaves are often used as mulch and for bedding in stables. They provide limited supplementary fodder for livestock under drought conditions in Africa. The use of leaves as fodder can be increased by improving palatability through breeding (Webb et al. 1967). It is used as a root stock for grafts of ornamental *Grevillea* species and cultivars because of its vigour and relatively disease-resistant root system. Kenya Forestry Research Institute (KEFRI), in 1990, has reported that the seed of the species was in such a high demand that it had not been able to supply enough to farmers.

The species occurs naturally on soil types with alluvium and basalts with pH range of 5.5–7.5 in soil textures varying from sand to sandy loam and loam to clay loam (Harwood & Owino 1992). *Grevillea robusta* does not tolerate swampy water-logged conditions although it grows well on river banks where water tables are often high but with lateral movement of ground water (Harwood & Booth 1992). Harwood (1989) also indicated that the tree did not perform well on heavy clay and acidic soils (pH 4.2). Most areas in western Kenya and some parts of central Kenya highlands have mainly acid soils with low levels of phosphorus and nitrogen (FAO 1986). This can make *G. robusta,* which is a phosphorus-sensitive plant, to harvest more phosphorus leading to toxicity especially in high levels of soil phosphorus. Studies using high phosphorus supply of 5mM caused an increase in leaf tissue including the epidermis (Karanja et al. 1999). Some members of Proteacea are susceptible to phosphorus and manganese toxicities (Kalpage 1967). Phosphorus toxicity has been shown to start as burning or discolouration of the tips and margins of older leaves. As the condition advances, the older leaves drop off giving a bare appearance to the base of the stem (Nichols et al. 1979).

Problems with boron deficiency and manganese toxicity have also been reported (Harwood 1989). Die-back of *G. robusta* in 1960 in tea plantation in Kenya was shown to be due to boron deficiency in the soil. Analysis of a sample of foliage showed leaves with high boron content (17 ppm) (Smith 1960) while an analysis of soil sample from the affected area showed an appreciably lower content of water-soluble boron (Venkataramani 1963). Recent studies have shown that the proteoid roots have the capacity of extracting nutrients in low levels and accumulating them to toxic levels.

Although *G. robusta* is attacked by pathogens and pests (Harwood & Getahum 1990), the reduction in vigour can predispose a multipurpose tree to pathogenic attack, which can destroy a whole regional population. In Africa, several pathogens have been associated with *G. robusta*. For example, in Kitui District in Kenya, a leaf blight disease caused by *Helminthosporium* spp. and *Colletotrichum corda* attacked seedlings in hot areas under excessive watering in 1984, a drought year in Kenya (Harwood 1989). Leaf spots caused by *Phyllosticta* and *Physalospora rhodina* have been reported in Malawi (Lee 1970).

Leaf spotting and abnormal growths were observed in *G. robusta* plantations in Kenya, mainly during the dry season at Yala and Kodera International Centre for Research in Agroforestry Forest (ICRAF) stations. The Yala site was mosaic, with unsightly appearances of unusual stunting, top drying and shrubby growths (rosettes) with some spotted leaves falling off to give the tree dark patches and sparse crowns. In one of the plots in Yala, the underside of the leaf was brown instead of the usual silky appearance. Other plots in the same area showed reasonably healthy trees. The trees at Kodera did not have the shrubby appearance but had very unsightly canopy especially during the dry season when attacked leaves fell off. This study investigated the cause of the leaf spot and the apparent decline of *G. robusta* plantings via macro- and microsymptom studies, reinoculations of seedlings and mineral analyses of the plant tissue and soil from various plots in order to identify agents of the forest decline.

MATERIALS AND METHODS

Plant materials

The *G. robusta* trees on ICRAF plots were planted in 1991 near the Yala market and in Kodera forest in Western province, Kenya. Most of the seedlings were from the Maseno ICRAF station which is near the Yala forest station. The Yala area has annual mean temperature from 20.5–21.7 °C, annual average rainfall from

1500–1900 mm and altitude from 1140–1400 m asl. The experimental woodlot is found on the banks of Yala river and was formerly a gold mine. It has 150 entries of provenances planted in a completely randomised design (CRD). The Kodera forest stands at an attitude of 1600 m at latitude 0° 35' and longitude 34° 38' E with average annual rainfall between 1400 and 1500 mm and annual mean temperature from 20.5–21.7 °C. The area has deep topsoil, is rich in organic matter and well drained (Jaetzold & Schmidt 1982) but the woodlot is planted on a slightly undulating landscape with reddish rocky soil. The plot is divided into three homogenous blocks with 42 accessions of *G. robusta* plants in a CRD which were spaced at 4 × 4 m.

Samples were representatively collected for disease investigation, and soil and tissue analyses. A macroscopic view was adopted to estimate the aesthetic value of the tree noting any gross abnormal characteristics such as dead twigs, wilted leaves, large cankers, wounds or even the presence of other organisms. This was carried out during the dry and wet seasons. Quantitative assessment of the disease was done according to Gatumbi et al. (1991) and microtome sectioning of diseased tissues was carried out using a modified version of the Jensen (1962) histological procedure.

Malt extract agar was used to grow the potential causal microbes from fresh but diseased leaves after 50% sodium hypochlorite surface sterilisation at 25 °C for 5 min. The isolates were developed into pure cultures and then used for pathogenicity tests following the Koch postulates. We inoculated plant seedlings from several sources in Kenya, i.e. K1 (Kakamega), K4 (Siaya), K7 (Kisii), K8 (Meru), K9 (Embu), Uasin Gishu and the Australian provenances.

Soil and plant samples

The samples of soil were collected at depths of 15–20 cm with the corresponding foliar material for manganese, phosphorus, potassium, calcium, nitrogen and soil pH analyses. A modified version of the Kjeldahl method (Cohen 1910) was used for nitrogen, while a modified Olsen method (Olsen at al. 1954) was used for potassium and phosphorus while other routine procedures were applied in analysis for the other elements. The amounts of proteoid roots in the plants were sampled by examining their accumulations at the bases of the trees.

RESULTS

Morphology of trees

The height of the trees ranged from normal tall to abnormally short (Figure 1). The slender trees had cracked bare stems with only few dead twigs while short trees had dead twigs with shrubby or rosette canopies reaching a height of 1–1.5m especially at the mid-plot. Drying of trees was also observed (Figure 1a). Much defoliation occurred during the dry season resulting in sparse canopies. In Kodera forest (Figure 1b), the trees showed almost normal morphology reaching a height of 15–20 m, with faint patches of brown leaves. Seedlings were also observed germinating under the canopy. During the dry season, the trees did not show any major morphological deviation from their appearance

(a) (b)

Figure 1 (a) A dwarfed tree in Yala forest and (b) normal trees in Kodera forest

during the wet season, except for an increase in the extent of tree defoliation.

In Yala forest, the leaf spots were found either on the tips or on the lamina of the leaves. The ones on the lamina were more conspicuous on the upper side. The appearance of the symptoms varied from spherical to irregular as shown in Kodera forest. Most of the lesions were found on either sides of the midrib. No conspicuous occurrences of tip lesions were observed. Some leaves showed rusty appearance with black spots on both sides of the leaf.

Disease assessment

Most of the plants showed leaf infection of 2.5–4% during the wet season and less than 2.5% during the dry season (Figure 2). The leaf infection in Kodera was less than 2.5% in both seasons. It was higher during the wet season especially in older leaves in position 4. The disease was more severe in Yala forest compared with Kodera.

Identification of isolated fungi

Microscopic examination of cultures and the use of modern mycological keys revealed that the black-coloured colony was *Phyllosticta* sp. A white colony known as *Pestalotia* sp. was commonly associated with it. The species in the *Phyllosticta* genus was confirmed to be the main cause. The other prominent microbe was a species from the *Pestalotia* genus which was found to be opportunistic. The fruiting bodies of *Phyllosticta* spp. showed the characteristic short-beaked pycnidia piercing the epidermis. It exhibited relatively fast growth in the form of black colony on fresh agar medium but produced pycnidia and other spores when the medium was drying. The mycelium spores were quite variant and hyaline. Some were aseptate, others were septated, thin walled, often gutted and of various shapes, somewhat ellipsoid, cylindrical, fusiform, pyriform or globose. The spores from the pycnidium were hyaline and oval in size 8–10 × 5–6 μm in length with short slender conidiophores. The mycelium showed twisted appearance reaching a diameter of 4 μm.

Microtome sections and pathogenicity tests

Most of the diseased leaf-tip microtome sections did not show the presence of pathogen. A study to verify this was initiated in which normal seedlings (see materials and methods) were subjected to soil from the most severely affected and least affected plots in Yala forest and grown for three months at another ICRAF station at Maseno during the rainy period. Leaf-tip drying and stunted plants were evident. Apparently normal seedlings growing on soil from Maseno ICRAF station showed leaf-tip drying and a faster leaf fall. Hence, it is hypothesised that the problem of tip drying is mainly abiotic rather than pathological. Microscopic examination of microtome sections of relatively young lamina lesions showed pathogenic attack was taking place. The attack seemed to follow the palisade cells first and then spread to adjacent mesophyll tissues. Intercellular mycelial spread was also

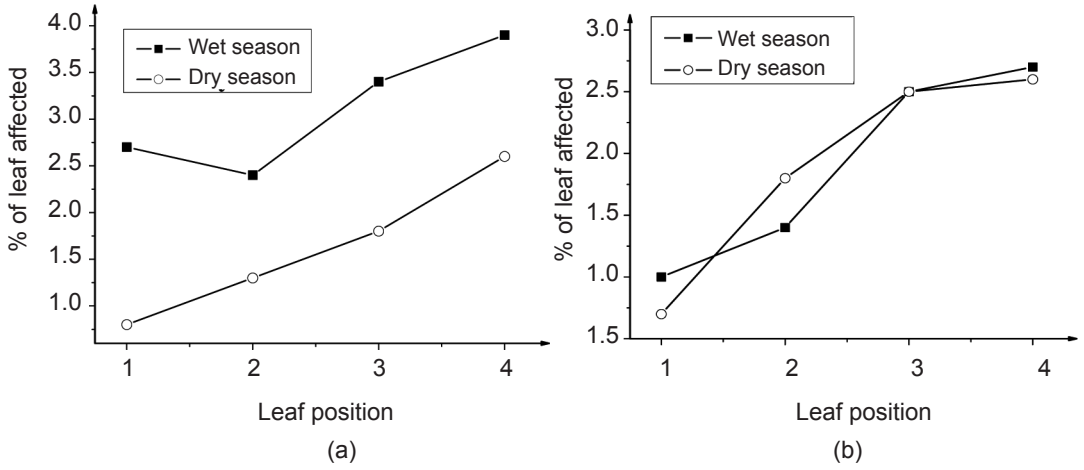

Figure 2 Differences in the percentage of leaf area affected against leaf position in (a) Yala forest and (b) Kodera forest. The leaf position shows the nearness of the leaf from the leaf apex (1) to four leaves in the branch (4).

observed on cut sections. Pathogenicity tests and observations on the fresh leaf-microtomed sections showed the twisting appearance of the *Phyllosticta* spp., unlike the smooth slender mycelium of *Pestalotia* spp.

Soil and tissue mineral evaluation

The analyses of soil and tissue were done and the results summarised in Tables 1 and 2. The values were carefully compared with the morphological appearance of the plants in the corresponding plots.

The plots in Yala had varied amounts of manganese concentration both in the leaf and soil (Figure 3). However, the highly morphologically affected trees seemed to be in plots with high amounts of manganese. Test seedlings from other locations (see materials and methods) grown in Maseno with soils from the Yala and Kodera plots showed similar stunted growths and leaf-tip drying. The soil and tissue manganese was positively correlated in Kodera (Figure 3).

DISCUSSION

Abiotic causes

The leaf spotting of *G. robusta* in the Western province of Kenya seems to be caused by a mixture of abiotic and biotic agents. The leaf spot symptoms of leaf tip necrosis and rosetting in Yala forest which was accompanied by chlorosis in Kodera forest are similar to symptoms due to phosphorus and manganese toxicity in Proteaceae (Shane et al. 2004). Another factor influencing the disease development is high mean temperature. The Kodera and Yala forest sites have annual mean temperatures between 20.5 and 21.7 °C, which is slightly higher than the normal requirement which is 13–20 °C (Booth & Javanovic 1988). However, the species grows well in higher temperature ranges in certain places (Harwood 1989). Hence, it is worthy to consider a breeding programme which can include hybridisation and grafting of *Grevillea* at temperatures raised by 2 °C in order to make Kodera and Yala forests and other sites more suitable. The Yala plot has acidic soil (average pH 4.8) while Kodera has an average pH of 6.3 (Table 2). *Grevillea robusta* normally requires pH 5.5–7.5 (Harwood et al. 1992). The Yala site (Table 1) has higher percentage of tissue phosphorus (0.07%) while Kodera has 0.06% which is normally within the range of healthy trees (Sagwal 1984). Higher levels of phosphorus in the medium increase its uptake in the plant (Thomas 1980). *Grevillea robusta* leaves analysed from healthy trees in India had 0.06% phosphorus, suggesting phosphorus toxicity in Yala plot. The Kodera site had a higher average potassium compared with Yala (Table 1). The analyses of soil and tissues were guided by the morphological appearance of

Table 1 Average values of minerals in leaf tissues

Site	%P	%N	%K	Mn (ppm)
Normal *G. robusta* requirement	0.06	0.53	0.42	281.9
Maseno	0.06	1.69	0.78	1627
Yala	0.07	1.40	0.95	635
Kodera	0.06	1.54	0.995	1002

P = phosphorus, N = nitrogen, K = potassium

Table 2 Average values of minerals in soil samples

Site	pH Soil: Water=1:2.5	TSN (g/kg)	TSP (g/kg)	EXK ($Cmol_c$/kg)	EXP (MgP/kg)	EXCa ($Cmol_c$/kg)
Maseno	5.40	0.50	0.20	0.200	7.70	1.90
Yala	4.82	1.17	0.33	0.261	15.45	3.05
Kodera	6.30	1.98	0.38	0.400	8.65	11.40

TSN = total soluble nitrogen, TSP = total soluble phosphorus, EXK = exchangeable potassium, EXP = exchangeable phosphorus, EXCa = exchangeable calcium

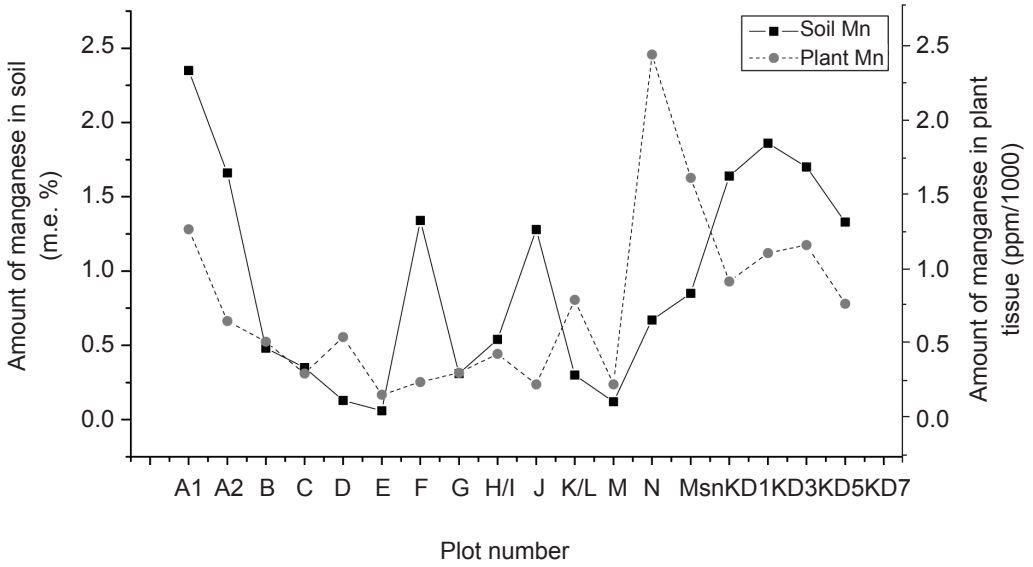

Figure 3 The correlation between soil and plant tissue manganese (Mn) shows that the Yala plot (A1, A2, B, C, D, E, F, G, H/I, J, K/L, M, N) is stochastic but the Kodera plot (KD1, KD3, KD5, KD7) shows a uniform relationship which is high. The Maseno (Msn) plot is also high.

plants in the field and seemed to follow closely the results of Thomas (1980). He postulated that high levels of potassium in the soil enhanced foliar potassium and mildly increased phosphorus uptake. Studies by Karanja et al. (1999) showed that 10% more calcium and high potassium resulted in increased leaf chloride and manganese but a decrease in total leaf iron and zinc. Earlier studies have shown that Proteaceae is adapted to low soil potassium of 0.8–8 mg kg^{-1} but some can tolerate until 70 mg kg^{-1} in the soil (Witkowski & Mitchell 1987). The exchangeable calcium in the plots showed that sites which had better morphological appearance of plants had also higher value of exchangeable calcium (EXCa). For example, Kodera Forest had an average EXCa of 11.4 $Cmol_c$/kg, Maseno had 1.90 $Cmol_c$/kg and Yala forest, 3.05 $Cmol_c$/kg. The most affected plots in Yala had the lowest values of EXCa of 2.0 and 1.9 while the least affected plots had the highest EXCa of 6.8 and 6.9. These results suggest a high possibility of exchangeable calcium deficit for *G. robusta*, especially in Yala and Maseno plots. The soil at the Yala plot has a lot of silt and is not well drained making the area unsuitable for the tree. The plot which is formerly a gold mine has mosaic soil composition. It has fast-growing grass which is usually cut manually, adding stress to the species.

The concern of a narrowed *Grevillea robusta* genetic base

The evidence that the genetic base of *G. robusta* has been narrowed through inbreeding (Harwood & Owino 1992) should be a caution to any further internal propagation of the local provenances. This could be evidenced from a survey which showed that an Australian seed produced about 30% more wood volume than the current trees in Kenya. Stress on the species can easily subject the trees to pathogenic attack as seen in Kitui District in Kenya in 1994, a drought year, when it was attacked by *Helminthosporium* spp. and *Collectotrichum* spp. (Harwood 1989). The fact that the Uasin Gishu provenance shows rather fast confinement of the disease after leaf inoculation indicates that some trees in Kenya still have good disease tolerance. However, it seems that tolerance is only possible when moisture, temperature or some minerals are not deficient.

Biotic causes of the leaf spot and epigenetic implications

The fungus *Phyllosticta* spp. was isolated from the leaf spots as the primary cause of the disease. However, the plants were found to be

in a seemingly complex abiotic stress which could have genetic and epigenetic implications. Epigenetic mechanisms usually provide extra information for a genome through cytosine DNA methylation, histone modification and nucleosome repositioning which can be reversibly inherited in plants. Abiotic stresses have unequivocally been proven to trigger inheritable epigenetic changes in plants (Zhao et al. 2007). Hence, generally the analysed data suggest that *G. robusta* in Kenya is not threatened by the leaf spot but is mainly due to the plant being grown in an unfavourable environment—high phosphorus, low calcium, poor drainage, high manganese, fast-growing grass, acidic soil and high temperature. The Maseno area attack is likely to be due to the species not being able to tolerate the low calcium and high temperature. High potassium and other abiotic minerals in interactions in the soil could contribute to mild attack. The symptomatic study of the mild attack in Kodera forest could be due to high temperature, probable narrowed genetic base and manganese toxicity.

RECOMMENDATIONS

Steps could further be taken to ameliorate the abiotic problem such as application of $FeSO_4$ which is known to bind soil PO_4^{3-} (Hawkins et al. 2008). The calcium:phosphorus ratio can be reduced by the addition of calcitic or dolomitic lime so as to reduce excessive manganese uptake. Hence, *G. robusta* still remains a species with great agroforestry potential. Research should be carried out on *G. pteridifolia* on the site. This is because this species performed better than *G. robusta* in re-vegetation of bauxite mining in India (Harwood 1989).

Proteoid roots have been observed to develop in soils with low phosphorus as they enhance nutrient uptake (Skene et al. 1996). Their absence in the Yala plot and small presence in Kodera has the implication of high amounts of phosphorus in the plots. The present situation of genetic erosion which is worsened by habitat quality declines in the tropics may persist unless the current inbreeding, habitat fragmentation and pollinator losses are not corrected in *G. robusta*. This can be buffered through establishment of seed farms in which optimum soil conditions are maintained and various exotic species from Australia are grown and genetically improved for seed production and distribution to the farmers. Recent studies have shown that even other common species are susceptible to the consequences of population genetic of habitat fragmentation just as is in the case of rare species (Honnay & Jacquemyn 2006). The lack of gene flow through mechanisms such as hindrances to seed dispersal, limited pollen transfer and large amount of inbreeding can cause genomic fragmentation leading to losses in genetic viability among former naturally distributed populations (Zhang et al. 2009). However, Kramer et al. 2008 suspect that most depressed recruitment of forest trees in fragments is due to reductions in pollination and dispersal agents rather than the loss of genetic variability. Studies in *G. robusta* in Kenya found very few aggressive and appropriate pollinators (Kalinganire et al. 2001).

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REFERENCES

Booth TDH & Javanovic T. 1988. Assaying natural climatic variability in some Australian species with fuelwood and agroforestry potential. *Commonwealth Forestry Review* 67: 27–34.

Cohen JB. 1910. *Practical Organic Chemistry*. MacMillan and Co, London.

FAO. 1986. *Efficient Fertilizer Use in Acid Upland Soils of the Humid Tropics*. FAO Fertilizer and Plant Nutrition Bulletin 10. FAO, Rome.

Ford HA, Barrett GW, Saunders DA & Recher HF. 2001. Why have birds of woodlands of southern Australia declined? *Biological Conservation* 97: 71–88.

Gatumbi RW, Kihurani AW & Skoglund LG. 1991. Severity of *Phomosis ipomoeae batata* (punita) in sweet potato in Kenya. Pp. 92–96 in *Proceedings of a KARI/CIP Technical Workshop on Collaborative Research*. 7–8 November 1999, Nairobi.

Harwood CE. 1989. Grevillea robusta: *An Annotated Bibliography*. ICRAF, Nairobi.

Harwood CE, Bell JC & Morang F. 1992. Isozyme studies on genetic variation and the breeding system in *Grevillea robusta*. Pp 165–176 in Harwood CE (Ed) Grevillea robusta *in Agroforestry and Forestry*. ICRAF, Nairobi.

Harwood CE & Booth TH. 1992. *Status of* Grevillea robusta *in Forestry and Agroforestry*. CSIRO, Canberra.

Harwood C & Getahum A. 1990. *Grevillea robusta*: Australian tree field success in Africa. *Agroforestry Today* 2: 8–10.

Harwood CE, Moran GF & Bell JC. 1997. Genetic differentiation in natural populations of *Grevillea robusta*. *Australian Journal of Botany* 45: 669–678.

Harwood CE & Owino F. 1992. Design of a genetic improvement strategy for *Grevillea robusta*. Pp 141–150 in Harwood CE (Ed) Grevillea robusta *in Agroforestry and Forestry*. ICRAF, Nairobi.

Hawkins H, Hettasch H, Mesjasz-Przybylowicz J, Przybylowicz W & Cramer MD. 2008. Phosphorus toxicity in the Proteaceae: a problem in post-agricultural lands. *Scientia Horticulturae* 117: 357–365.

Honnay O & Jacquemyn H. 2006. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21: 823–831.

Jaetzold R & Schmidt H. 1982. *Farm Management Handbook of Kenya*. Volume II. Ministry of Agriculture, Kenya.

Jensen WA. 1962. *Botanical Histochemistry*. WH Freeman and Co, San Francisco.

Kalinganire A, Harwood CE, Slee MU & Simons AJ. 2001. Pollination and fruit-set of *Grevillea robusta* in western Kenya. *Austral Ecology* 26: 637–648.

Kalpage FSCP. 1967. Manganese in soils and tea under *Grevillea* shade. *Tropical Agriculture* 44: 209–214.

Karanja NK, Mwendwa KA & Zapata F. 1999. Growth response of *Grevillea robusta* A. Cunn. seedlings to phosphorus fertilization in acid soils from Kenya. *Biotechnology, Agronomy, Society and Environment* 3: 57–64.

Kramer AT, Ison JL, Ashley MV & Howe HF. 2008. The paradox of forest fragmentation genetics. *Conservation Biology* 22: 878–885.

Lande R. 1988. Genetics and demography in biological conservation. *Science* 241: 1455–1460.

Lande R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *The Naturalist* 142: 911–927.

Lee RF. 1970. *A First Checklist of Tree Diseases in Malawi*. Research Record 43. Forest Research Institute of Malauri, Zomba.

Midgley SJ. 1983. An *Assessment of the Current Use and Potential of Australian Woody Species in Conventional and Non-conventional Forestry Systems in Kenya*. CSIRO, Canberra.

Nichols DG, Beardsell DV & Knoxfield HRI. 1979. Phosphorus toxicity in Australia native plants. *Seed and Nursery Trader* 77: 25.

Olsen S, Cole C, Watanabe F & Dean L. 1954. *Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate*. USDA Circular No. 939. US Government Printing Office, Washington DC.

Sagwal SS. 1984. Silver oak: a tree of many uses. *Indian Farming* 34: 29–32.

Shane MW, Mccully ME & Lambers H. 2004. Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea protrata* (Proteaceae). *Journal of Experimental Botany* 55: 1033–1044.

Skene KR, Kierans M, Sprent JJ & Raven JA. 1996. Structural aspects of cluster root development and their possible significance for nutrient acquisition in *Grevillea robusta* (Protaceae). *Annual Botany* 77: 443–451.

Smith AN. 1960. Boron deficiency in *Grevillea robusta*. *Nature* 186: 987.

Thomas MB. 1980. Phosphorus response of Proteaceae and other nursery plants in containers. *Royal New Zealand Institute of Horticulture Annual Journal* 8: 21–33.

Venkataramani KS. 1963. Boron deficiency in the silver oak. Pp 70–71 in *Annual Administration Report*. United Planters Association of South India, Devarshola.

Webb L, Tracey JG & Haydock KP. 1967. A factor toxic to seedlings of the same species associated with living roots of the non-gregarious subtropical rainforest tree, *Grevillea robusta*. *Journal of Applied Ecology* 4: 13–25.

Witkowski ETF & Michell DT. 1987. Variations in soil phosphorus in the Fynbos Biome, South Africa. *Journal of Ecology* 75: 1159–1171.

Zhang JF, Kimatu JN, Guo WL & Liu B. 2009. Habitat fragmentation causes rapid genetic differentiation and homogenization in natural plant populations—a case study in *Leymus chinensis*. *African Journal of Biotechnology* 15: 3440–3447.

Zhao X, Chai Y & Liu B. 2007. Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. *Plant Science* 172: 930–938.

