Variation in Seedling Density, Herbivory and Disease Incidence among Seedling Stages and Mother Prunus *Africana* Trees Growing on Varying Microsites in a Transitional Rainforest in Kenya

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Abstract
The study was carried out at Kakamega Forest which is generally considered to be the easternmost limit in today's climate of the lowland Guineo Congolean rainforest of central Africa. Faunally and florally, Kakamega is dominated by central African lowland species, but due to its elevation (1,400-2,300 meters (4,000-7,000 ft.) and proximity to the formerly contiguous Nandi Forests it also contains well-represented highland elements and is thus unique, thus, it is a significant island of biodiversity that has developed along its own unique evolutionary course for thousands of years and which shows a high level of endemism. The objective of the study was to determine how herbivory and disease incidence vary among seedling stages and trees growing in varying micro sites. The study began at the end of the fruiting season and start of germination of seeds. A natural stand was identified where *Prunus africana* was abundant because the species density. The results indicated that seedling abundance, disease incidence and herbivory varied among trees and microsites.
Keywords: Herbivory, Disease incidence, Regeneration, Prunus

Introduction

Herbivory is the term applied to the animal consumption of any plant material, and it ranges from 0% up to 100% leaf surface area removed (defoliation at this level of severity), but it is a common convention to use herbivory to indicate folivory (Lowman, 1997) Insects are the most abundant herbivores although birds and mammals also play important roles (Schuldt, *et al*., 2010).). Insect herbivores damage plants in a variety of ways, some being more obvious than others (Björkman,*et al*., 2011).

Disease on the other hand is a disturbance in the normal physiological functioning of a plant, has many causes, and exhibits an array of appearances and results (Manion, 1981; Rohrs-Richey, 2010). Any agent that causes disease is a pathogen and may either be biotic such as fungi or abiotic such as air pollution. Some pathogens are parasites, but not all parasites are pathogens. Any organism that lives on and derives nutrients from another organism is a parasite, but only those parasites that cause a disruption in the normal physiological function of the host are classified as pathogens (Manion, 1981). Biotic diseases, because infectious agents cause them, usually show a clumped distribution pattern of diseased individuals (Horsfall & Cowling, 1977; Narayanasamy, 2010). Inoculum produced by diseased individuals is most concentrated around the diseased individuals, thereby contributing to a higher incidence of disease in localized areas. Only with initial infection caused by inoculum dispersed from a distance does the distribution of disease approach randomness (Manion, 1981). Topographic features that produce moisture or temperature conditions favourable for inoculum production, dispersal and infection may contribute to the clumped disease distribution patterns typical of biotic diseases. Abiotic diseases are usually random in a population except when the agent is distributed in a non-random manner (Narayanasamy, 2010)**.**

Herbivory and attack by diseases play an important role in organisation of plant communities as well as being selective forces in the evolution of plant secondary chemicals and plant morphology (Carmona, *et al*., 2011). Several workers (Brown, 1982, 1985; Brown *et al.* 1987) have experimentally shown that natural levels of insect herbivory and diseases can have substantial effects on species richness, plant cover and seedling establishment as well as influencing the growth, survival and reproduction of individual species. However, the effects on host plants depend on a number of factors, including host plant characteristics such as age, size and vigour (Johnson, 2011; Macel, *et al*., 2010).

Regeneration of tree species can be characterised by the scale of disturbances and environmental heterogeneity to which the species responds, and by the spatial relationships between adults and juveniles (Grau, 2000). In the absence of large-scale catastrophic disturbances, regeneration dynamics is strongly influenced by endogenous factors including density-dependent mortality of seed and seedlings due to pathogens, herbivores and predators. Studies on such individual factors can reveal much about the variables determining success at different levels (Rodríguez‐Pérez, *et al*., 2012) Changes in the number of individuals and their spatial pattern during the transition from seeds to saplings have important implications for the fitness of the parent, the size and the genetic and spatial structure of populations and ultimately, species diversity and pattern within the community.

The broad objective of this study was to determine how herbivory and disease incidence vary among seedling stages and among trees growing in varying micro sites.

The specific objective was to determine the relationship between herbivory, disease incidence, and seedling abundance. It was hypothesised that there was no significant differences between herbivory, disease incidence and seedling growth in different microsites.

Methods

This study was carried out in a natural forest stand in Kakamega forest reserve, and it began at the end of the fruiting season and start of germination of seeds. A natural stand in this case is defined as a natural forest where most of the species present where not planted except for limited enrichment planting. In Kakamega the peak flowering season of *Prunus afri*cana occurs in the months of June to August, although sporadic flowering occurs all year round. Based on information obtained from the forest office, a natural stand was identified where *Prunus africana* was abundant because the species density is generally very low. Once the stand was identified, a random point was chosen in the centre of the stand. A point-centred quarter sampling approach was adopted (Bullock, 1996). Two perpendicular straight lines, which cross each other on the sample point, were measured out. This created four sampling units (quarters). In each quarter, and starting from the random point, the nearest mature and seeding *Prunus africana* tree was identified. Thereafter, a second tree nearest to the first one but at least fifty meters from the first one and within the quarter was identified. The same procedure was used in the other quarters until five trees were identified in total, at least one from each quarter. For a tree to qualify to be selected it had to be seeding and at least fifty meters from the last sampled tree. For each tree, DBH, height and general information of the microenvironment in which the tree was growing was recorded, and is presented on table 1.. Study tree number one was located at the edge of the forest were it grades into open grassland. The rest of the trees were located in closed forest, at least fifty meters from the forest edge.

Table 1. General information associated with each parent tree

Tree No.	DBH (cm)	Height (m)	Location within forest	Other information
1	41	29	Edge	Open – no undergrowth
2	46	31	Inside	Average density undergrowth
3	39	30	Inside	Average density undergrowth
4	56	35	Inside	Average density undergrowth
5	79	37	Inside	Average density undergrowth

Key. 1. Open –(no undergrowth) – less than 10% undergrowtg per unit area
2. Average density undergrowth – 10-50% undergrowth per unit area
3. Dense undergrowth – more than 50% undergrowth per unit area

To determine how herbivory and disease incidence vary among seedling stages and among trees growing in varying micro sites, a modified Adaptive Cluster Sampling design was used in the study. The procedure is described by Thompson (1991, Acharya *et al*. (2000) and Levy, *et al*., 2013).

Prunus africana seedlings undergo four distinct stages that can be recognized on the basis of the fixed number of leaves present at each stage (Tsingalia, 1989). Seedlings were counted in the 1-m^2 quadrats and assigned to the relevant category based on number of leaves and height as an indicator of cohort age.

- 1- seedlings < 10 cm tall, with a single apical meristem without any leaves (Clark & Clark, 1985). Seedlings in this category would be less than three months old.
- 2- seedlings ≥10 < 25cm tall**,** with two green leaves but without an apparent meristem. Seedlings in this category would be more than three months old and less than one year old.
- 3 - seedlings ≥25 **<** 50 cm tall, with three to four leaves and a distinct meristem. Seedlings in this category would be more than one year old and up to three years old.
- 4-– seedlings ≥ 50 **<** 100 cm tall, with more than four leaves and a distinct meristem. These seedlings would be about three to five years old.

Each seedling was inspected for evidence of disease and/or herbivore attack. Any seedling without a meristem, or which had lost its original colour through decay, or with brown patches or signs of rotting, was considered a victim of disease attack. Seedlings with parts of their foliage missing or with holes in their foliage were considered victims of herbivory.

Data analysis
Disease incidence and herbivory as percentages of the total count of seedlings for the quadrat and tree concerned was used to asses variation in disease incidence and herbivory. Percentages of disease incidence and herbivory were arcsine transformed before ANOVA to normalize distribution.

Karl Pearson's correlation was undertaken to test the relationship between herbivory, disease incidence, and seedling abundance; one-way ANOVA tests where done to decide whether significant variations occurred in prevalence of seedling attack by disease and herbivores among: (i) stages of seedling development; and (ii) among trees growing on different microsites. When significant variations were detected, Duncan's multiple range tests were done to compare variations among levels.

Results
Variation in seedling abundance by stages and among trees
Table 1 shows show total counts and percentages of seedlings of all stages at each of the five study trees. The data shows that the density of seedlings varied among stages and among study trees growing on varying microsites. The number of seedlings ranged from 1,683 (33.7/m^2) to 8,847 (176.9/m^2), with a mean of 6,598 (132/m^2). Tree number 1 had lower seedling abundance than the rest of the trees. There were significantly (ANOVA, P< 0.001) more stage two seedlings and fewer stage four seedlings, and this trend was maintained whether one considers the individual trees or the overall abundance of seedlings.

Table 1. Total counts of seedlings of all stages at each of the five study trees.

Seedling stage →		**Stage 1**		**Stage 2**		**Stage 3**		**Stage 4**		
Height (cm)→		**<10**		**≥10 ≤25**		**≥25 ≤50**		**≥50 ≤100**		
Tree ↓	Area (m^2)	Number	%	Number	%	Number	%	Number	%	Total
1	50	155	9.21%	1393	82.77%	115	6.83%	20	1.19%	1683
2	50	795	11.39%	5840	83.67%	331	4.74%	14	0.20%	6980
3	50	246	3.01%	7710	94.40%	209	2.56%	2	0.02%	8167
4	50	283	3.87%	6840	93.51%	180	2.46%	12	0.16%	7315
5	50	375	4.24%	8335	94.21%	131	1.48%	6	0.07%	8847
Total	250	1854	31.72%	30118	448.56%	966	18.08%	54	1.65%	32992
Mean		371	6.34%	6024	89.71%	193	3.62%	10.80	0.33%	6598
Stdev.		249.91	3.72%	2753.60	5.95%	85.73	2.16%	7.01	0.49%	2843.28

Variation in seedling herbivory by stages and among trees growing in different microsites
Table 2 shows total counts and percentages of seedlings of all stages attacked by herbivores at each of the five study trees. Percentage herbivory increased gradually from seedlings of stages one to four, and was significantly (ANOVA, P< 0.001) higher on seedlings of stage three and four. Herbivory varied among trees and was higher in seedlings of tree number one as compared to the rest.

Table 2. Total counts of seedlings of all stages attacked by herbivores at each of the five study trees.

Seedling stage →		**Stage 1**		**Stage 2**		**Stage 3**		**Stage 4**			
Height (cm)→		**<10**		**≥10 ≤25**		**≥25 ≤50**		**≥50 ≤100**			
Tree ↓	Area (m^2)	Number	%	Number	%	Number	%	Number	%	Total	%
1	50	33	21.29%	310	22.25%	47	40.87%	9	45.00%	399	23.71%
2	50	45	5.66%	979	16.76%	111	33.53%	7	50.00%	1142	16.36%
3	50	8	3.25%	1433	18.59%	106	50.72%	2	100.00%	1549	18.97%
4	50	28	9.89%	1297	18.96%	45	25.00%	1	8.33%	1371	18.74%
5	50	14	3.73%	1640	19.68%	69	52.67%	0	0.00%	1723	19.48%
Total	250	128.00	43.83%	5659.00	96.24%	378.00	202.79%	19.00	203.33%	6184	
Mean		25.60	8.77%	1131.80	19.25%	75.60	40.56%	3.80	40.67%		
Stdev		14.84	7.48%	518.41	1.99%	31.52	11.63%	3.96	39.77%		

Variation in disease incidence by seedling stages and among trees growing in different microsites
Table 3 shows total counts and percentages of seedlings of all stages infected by disease at each of the five study

trees. Disease incidence on seedlings decreased from seedlings of stage one to three, and was significantly (ANOVA, P< 0.001) lower amongst seedlings of stages three and four. Disease incidence was lowest in tree number one as compared to the rest . Although seedling mortality was not studied, it was observed that most of the disease infected seedlings died within 2 weeks.

Table 3. Total counts of seedlings of all stages infected by disease at each of the five study trees

Seedling stage →		**Stage 1**		**Stage 2**		**Stage 3**		**Stage 4**			
Height (cm)→		**<10**		**≥10 ≤25**		**≥25 ≤50**		**≥50 ≤100**			
Tree ↓	Area (m^2)	Number	%	Number	%	Number	%	Number	%	Total	%
1	50	40	25.80	174	12.49	9	7.83	3	0.38	226	13.43%
2	50	329	41.38	1695	29.02	38	11.48	1	7.14	2063	29.56%
3	50	127	51.63	2202	28.56	21	10.05	1	50.00	2351	28.79%
4	50	95	33.57	1829	26.74	19	10.56	2	16.67	1945	26.59%
5	50	101	26.93	2197	26.36	30	22.90	2	33.30	2330	26.34%
Total	250	692	179.31	8097	123.17	117	62.82	9	107.49	8915	
Mean		138.4	35.862	1619.4	24.634	23.4	12.564	1.8	21.498		
Stdev		111.16	10.79	838.43	6.88	11.06	5.93	0.84	20.17		

Relationship between herbivory, disease incidence and seedling density

Disease incidence and herbivory were inversely correlated along the seedling stage gradient (**Fig 1**). The smaller the seedlings the higher was the disease incidence, but lower herbivory. As the seedling size increased, however, damage due to herbivores dominated. Both disease incidence and herbivory were positively correlated with seedling density (r^2=0.996 and 0.990 Resp.).

Discussion

The data presented indicate that mortality of seedlings in *Prunus africana* is very high, as demonstrated by the decreased abundance of seedlings of stages three and four; the number of seedlings present at each stage decreases as the seedlings develop from one stage to another. Stage one seedlings (<10 cm tall) are clearly of a different cohort from stage four (≥50<100cm tall), therefore by stage 4 there are very few seedlings left that will be recruited as adults. Fungal pathogens and herbivores could be the major cause of mortality in natural populations of *Prunus africana* seedlings, although one cannot preclude the actions of other agents of mortality such as fallen debris and inadequate light. A number of past studies have shown herbivore-induced mortality of seedlings in other tropical forests (e.g. Fried et al., 1988, 80% and Howe, 1990, 51%). In these studies seedlings were marked and re-assessed on different dates.

Seedlings of parent trees located at the forest edge and deep in the forest which differed in microclimate exhibited different densities, levels of herbivory and disease incidence. This indicates that micro-habitat plays a crucial role in determining seedling density, and levels of herbivory and disease. A higher percentage of stage four seedlings were found at tree one. This tree was located at the forest edge where the forest grades into the grassland. The micro-environment under this tree might have been more suitable for herbivores and less suitable for pathogen infestation. Saplings were also observed in gaps within the forest that were not within the sampling areas. Low disease attack in the forest edge may account for the abundance of stage four seedlings in tree number one and in forest gaps. The presence of a higher percentage of stage four seedlings at the forest edge, and of saplings in gaps indicates the importance of seedlings escape from disease attack

The major herbivores in this case were probably blue headed monkeys, and Goliath beetles, which were encountered on several occasions in the study sites. Consequently, abundance of these herbivores is critical to seedling establishment, and hence regeneration of *Prunus africana.* Where damage is minimal, the ability of the seedlings to re-sprout would help them to withstand or recover from such damage as observed in some of the seedlings in this study.

Mwanza *et al.* (1999) found that leaves of *Prunus africana* collected from wildings in natural forests in Kenya were heavily infected with a leaf spot disease caused by *Colletotrichium gloeosporioides*. When infection was severe, the pathogen caused premature leaf fall and die-back of the leader shoot. Although the samples were not analysed for this pathogens, they are a possible cause of disease in this study, given that Mwanza *et al.* (1999) studied seedlings including those collected from Kakamega. Disease incidence varied inversely with herbivory across the seedling stages. Higherlevels of herbivory in stage four seedlings might be due to time effect and because there are more leaves to show damage. Damage due to disease falls probably because of mortality effects and increased isolation of individuals (lower density). Seedlings are known to be less vulnerable with time (Populer, 1978), as they undergo cell wall thickening and lignification (Walker, 1969). It is for this reason that seedlings might be prone to disease attack at earlier stages, later however, as the seedlings mature, they might become less favourable to pathogens but suitable for herbivores (Coley, 1983). However, it is not known at what stage seedlings of *Prunus africana* get lignified (Bell, 2012).

Conclusion

The results indicated that seedling abundance, disease incidence and herbivory varied among trees. Tree number one who was located at the edge of the forest experienced a significantly higher level of herbivory, lower level disease incidence and low seedling density as compared to the other trees. The variation in herbivory and disease incidence in the five study trees may be due to: (i) variation in the micro-environmental conditions in the vicinity of the various trees; (ii) variation in trees in their herbivore and pathogen populations; (iii) variation in seed crop among the trees; and (iv) genetic variation among trees in their susceptibility to herbivore and pathogen attack.

The regeneration of *Prunus africana*, the results of the study showed broader implications for understanding population and community dynamics in tropical forests. Population growth depends not only on juvenile survival, growth and spatial distribution, but also on the recruitment of new juveniles into the population. The quantity of this new recruitment is a complex outcome of many factors acting to reduce the potential seedling production

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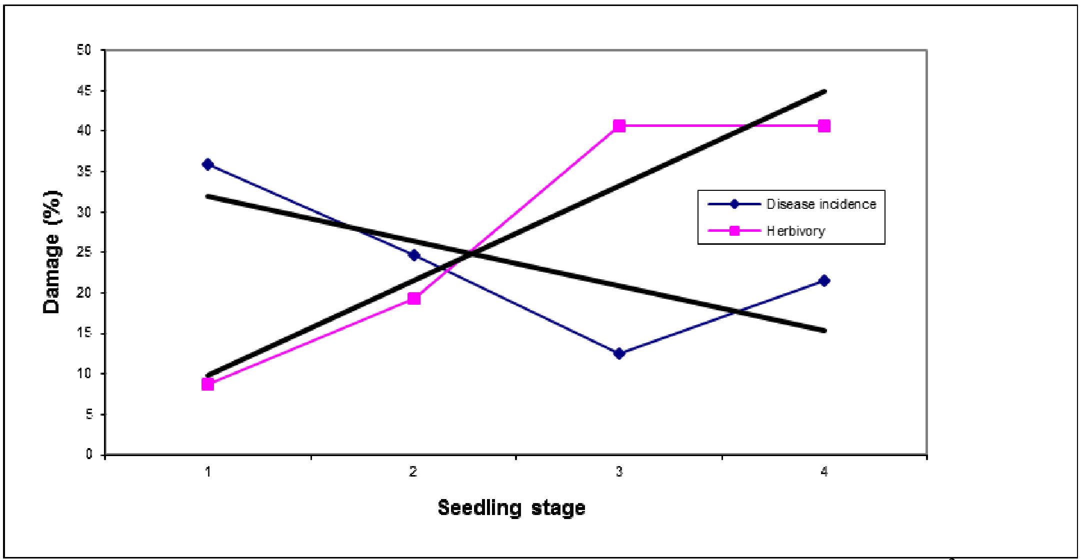

Figure 1. Relationship between percentage herbivory and percentage disease incidence (m^2) across the four seedling stages. Damage levels were calculated as the percentage of the number of seedlings at the stage concerned.