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Full Length Research Paper

Screening Sclerocarya birrea provenances on pests and diseases: A step towards domestication

Chicco D. M. Kayange^{1*}, Nyirenda S. P.², Mhango J.³, Njera D.³, Manda C. S.⁴, Rudoviko M.⁵ and Kaponya I.⁶

¹Department of Land Resources Conservation, Mulanje District Agriculture Office, P. O. Box 49, Mulanje, Malawi.
 ²Department of Agricultural Research Services, Bvumbwe Research Station, P. O. Box 5748, Limbe, Malawi.
 ³Department of Forestry and Environmental Sciences, Mzuzu University, P/Bag 201, Luwinga, Mzuzu 2, Malawi.
 ⁴Department of Environmental Affairs, Mulanje District Council, Private Bag 9 Mulanje, Malawi.
 ⁵Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.
 ⁶Blantyre Agriculture Development Division, Private Bag 379, Blantyre, Malawi.

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Sclerocarya birrea is a famous wild fruit tree species which constitutes an important agro ecological system of the global ecosystem. It is one of the widely spread miombo woodland fruit trees species within sub Saharan Africa. An international provenances trial of 21 genotypes evaluated the plant phenology thus including, growth, adaptability (survival), fruit productivity and quality to select genotypes with superior traits well adapted to local conditions for domestication. This study aimed at screening provenances to pests and diseases as advancement to domestication. The provenance trial was laid out in a randomized complete block design (RCBD) with four replicates. Each treatment was represented by 21 provenances including one bulked from Tanzania. There were 333 families assigned in each block and repeated four times. Each provenance was assigned in each row of 20 families as treatments. These provenances and families were randomly assigned to each plot and blocked. The spacing was 4 m between families and 5 m between provenances. Data was collected on infestation pressure to pests and diseases and analyzed. There were significant differences in mean infestation of defoliating and skeletonizing insect pests between provenances (P≤ 0.001). Magunde, Mangochi, Chikwawa, Marracuene and Rumphi provenances were more significantly susceptible to defoliators than the rest of provenances. The results showed that there is variation on levels of susceptibility and tolerance to diseases between provenances. Therefore, when promoting these provenances for domestication, pest and diseases management to be considered as an important selection tool hence propagate and safeguard promising elite provenances as potential genotypes for social and economic gains.

Key words: Tolerance, elite, susceptibility, genotypes, infestation.

INTRODUCTION

Sclerocarya birrea forms part of the famous wild fruit trees which constitute an important agro ecological systems all over the world (Chivandi et al., 2015). It is

one of the widely spread miombo woodland fruit trees species within sub Saharan Africa (Mkwezalamba et al., 2015). As such, it is forming part of the world famous largest ecological Miombo woodland ecosystem even though its health and biodiversity is undergoing a serious threat (Tubby and Webber, 2018; Sniezko and Koch, 2017). Despite the enormous benefits accrued from the species, there are several risks associated with its existence and biodiversity. Apparently, the most distinguished risks are insect pests and pathogens. Pests and pathogens are often suggested as exacerbated by climate change which influences genetic and epigenetic changes according to ecological adaptations (Chaudhary, 2013; Tubby and Webber, 2018; Sniezko and Koch, 2017). Generally, pests and pathogens abundance and distribution are influenced by change in the environment condition (Foggo et al., 2003). In this case, climatic variations, such as temperatures according to Hendry et al. (2005) escalate pest and pathogen development by inducing stress to crops and making them favorable for pests and disease development. Based on the same understanding, Street et al. (2011) reported that the shift of a plant from its initial agro ecological zone has the potential to attract insect and pathogen attack. The possibility is that of alteration in the chemistry of the plant such as concentration of soluble carbohydrates (Crispo et al., 2010). Therefore, domestication, which is the process of adapting a wild plants or animals for human use has the potential of accelerating the pests and diseases. Therefore, the process of that transition of wild progenitor species into modern elite cultivars through domestication has high risks of bringing new pests and diseases. In response to such selection, most plant species have exhibited changes in their phenotypic characteristics (Friedman et al., 2011) which carefully could have an acceleration to new pests and pathogens (Reddy, 2013; Lok et al., 2018). It is from theories of Darwin (1857) that phenotypic variation between wild and domesticated plants presents an opportunity to generate insights into evolution principles of using the morphologically variable antecedent and descendant taxa (Chaudhary, 2013). This concept was demonstrated through realization of natural selection pressure which is adaptation under human selection often leading to unexpected and unexplained departures from predicted phenotypes. Generally, these traits include, enhanced yield, enhanced apical dominance, reduced seed dormancy, perennial to annual habits and relatively susceptible to pests and diseases (Chaudhary, 2013; Fu et al., 2014).

It is from this stand point that *S. birrea* also called *Marula* has gone into a series of studies in preparation to bringing into agriculture. Based on its importance, *S. birrea* is known for social and economic benefits among many rural communities in Eastern and Southern Africa

(Akinnifesi et al., 2004; Mkwezalamba et al., 2015). The fruit has been a reverence for provision food security by meeting nutritional and economic needs for all among the rural communities (Akinnifesi et al., 2004) Despite such economic gains, there is a threat to the species of losing its ecosystem services and genetic diversity. In this regard, there is a requirement to make an advancement towards selecting elite cultivars which can be conserved and managed by domestication to benefit humans through social and economic gains (Mkwezalamba et al., 2015). While selection is largely dependent on phenotypic variations, there is high possibility that amongst individuals of the same population, there are differences in susceptibility and tolerance to pests and diseases due to alterations as a result of interaction between and among genes and the environment (Chaudhary, 2013).

According to Tubby and Webber (2018) suggest that the level of pest and disease infestation easily escalates when a crop is introduced to a new environment. A shift of an indigenous fruit tree from its natural environment to monoculture is likely to experience and introduce new pest pressure due to loss of its buffering capacity it had from natural systems (Reddy, 2013; Lok et al., 2018). Therefore, in an event where *S. birrea*, a wild tree crop is to be introduced into agriculture, information regarding the insect pests and diseases must be researched, which at this point remain limited (Msukwa et al., 2016). The limitation to such information such as insect pest and pathogen (Msukwa et al., 2016) has necessitated this study.

MATERIALS AND METHODS

Study location

The study was conducted at Palm Forest Reserve in Mangochi, Malawi. This is an experimental site where an international provenance trial of 21 provenances of *Sclerocarya birrea* was established under World Agroforestry Center (ICRAF) through Forestry Institute of Malawi (FRIM) in the Department of Forestry. This trial was established in 1999 with seed collection from different countries in Africa. It is located along the latitude and longitude of 14°28' South and 35°14' East of the prime meridian, respectively. The area lies at an altitude of 200 m above sea level with an average annual rainfall of 800 to 1200 and mean annual temperature of 24.0°C. The site is generally flat and susceptible to flooding. Soils are predominately sandy to loamy soils with pH 4.5 to 7 (Chivandi et al., 2015; Msukwa et al., 2016). The natural vegetation comprises of palms (*Hyphaene spp*) and *Acacias* species (Msukwa et al., 2016).

*Corresponding author. E-mail: <u>chiccokayange2022@outlook.com</u>.

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Experimental material and data collection

The twenty-one (21) provenances were raised at the nursery site of Forestry Research Institute of Malawi (FRIM). The seed was sown directly in standard polythene tube (15 cm × 10 cm) filled with soils collected within the Miombo woodland (Msukwa et al., 2016; Nyoka et al., 2015). Seed lots from Zambia, Tanzania, Namibia, Mali and some from Malawi were sown in February, 1998 as opposed to provenances from Mozambique Zimbabwe and Malawi particularly from Rumphi sown in August, 1998 (Msukwa et al., 2016). Makadaga-Mbarali, Nyamahanga-Iringa and Kigwa-Tobora from Tanzania which were identified from a subspecies multifoliolata did not produce enough seedlings due to poor germination as a result it were not included in the field experiment (Msukwa et al., 2016). Therefore, those provenances with relatively few seedlings were bulked and these include Mkata-Morogoro, UbeNa-Bagamoyo, Chalinze-Bagamoyo, Kigwe-Dodoma, Mialo-Kondoa. and Mandimu-Singida. It was only Magamba-Turiani, a Tanzanian provenance which had enough seedlings that was therefore assigned as sole treatment (Table 1). Consequently, the trial was established with 21 individual provenances and one bulked seed lot under study.

Study design

The provenance trial was laid out in a randomized complete block design (RCBD) with four replicates. Each treatment was represented by 21 provenances including one bulked from Tanzania. There were 333 families assigned in each block and repeated four times. Each provenance was assigned in each row of 20 families as treatments. These provenances and families were randomly assigned to each plot and blocked. The process was repeated in families less than 20 in numbers within a row to have a full number of trees in a line plot and was equally assessed. The spacing was 4 m between families and 5 m between provenances.

Data collection

Data was collected from November 2018 to October 2019 at 19 years age of *Sclerocarya birrea* International Provenances trial in Mangochi. Data on insect pests and diseases were collected specifically on foliage, stem and fruit damage. The level of pest and disease infestation were also assessed on trunk, foliage and fruit (Burks and Brandl, 2005). The sampling techniques of insect pest deployed where both absolute and relative. Absolute sampling technique implies to insect pest per tree crop were assessed as it occurred as opposite to relative sampling technique where insect pests were collected using apparatus such pheromone traps to infer data over time and space (Litsinger et al., 1998).

Insect incidence and damage assessment

Insect pest damage scores were done on foliage, stem and fruit following (Burks and Brandl, 2005) protocol. The assessment was grouped into two phases namely; at leaf flushing and fruiting. Insect pest scoring was done early in the morning and late in the afternoon were most of the leaf feeding insects are at peak. All the trees within the trial were assessed except for the Tanzanian provenance which was bulked. Fruits were scored depending on the type of damage caused by insect pests. Rating scale of leaf and stem damage of *S. birrea* were investigated based on Obeng et al. (2015). The assessment mode was rated at the scale of 1 to 5 as presented in Table 2. The percentage damage was derived from leaf damaged per provenances in relation to the entire tree canopy. Data was collected on canopy visually based on individual family. Where a family where repeated, an average score was determined and recorded.

Aphid infestation and damage were assessed from each family in a provenance monthly. The targeted part of the plant was the active growing point where there were flesh leaves. The presence of aphid was recorded based on visual observations. Each colony size was visually scored on a scale of 1 to 5 points as stated in Table 3. The number of plants infested against severity of infestation was recorded per plant in plot in accordance with Litsinger et al. (1998) protocol

Assessment of disease incidence and severity

Incidence and severity of diseases were done in accordance with of Borges et al. (1997) study. In this, disease incidence was measured as frequency of diseased or damaged individual or parts proportional to plant unit. While disease severity was based on the amount of plant tissue disordered and expressed as a percentage (Borges et al., 1997). The disease incidence and severity score was based on the rating of 1 to 4 (Table 4 adopted from Manandhar et al., 2016).

Mathematically disease incidence (%) was calculated as follows:

Disease Incidence (%) = Total number of plants observed × 100

Observed lesions on infected leaves were estimated physically measuring the diseased parts using the scoring scale as depicted in Table 5. Further identification on diseases samples were collected for culturing at Byumbwe Agricultural Research Station.

Statistical analysis

Data on insect and disease abundance and severity were collected and subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS). The significance level was set at 5% and the mean values that showed significant differences were separated using the Fisher's least significant difference (Lsd).

RESULTS

Screening of provenances on insect pests' infestation (defoliators, skeletonizers and aphid)

There was a significant difference in mean infestation of defoliators, skeletonizers and aphid pests between provenances at P \leq 0.001 in 2018/19 and 2019/20 seasons (Table 6). Magunde, Mangochi and Rumphi provenances were significantly more infested with defoliators than the rest of provenances in during the study period and Mzarabani the least defoliated (Table 6). The provenances which were significantly skelelotinized were Mzarabani, Maraccune and Magunde than the rest during flushing. Chikwawa, Rumphi and

Provenance	Code	No. of families	Latitude	Longitude	Elevation (m)
Malawi		· · · ·		· · ·	· · · ·
Chikwawa	ML1	10	16°46'S	35°17'E	100-300
Mangochi	ML2	20	14°02'S	34°53'E	200-600
Ntcheu	ML3	19	14°39'S	34°46'E	300-600
Rumphi	ML4	20	10°59'S	33°45'E	900-1 200
Mozambique					
Marracuene	MZ1	17	25°58'S	32°95'E	0-200
Magunde	MZ2	20	24°95'S	32°92'E	0-200
Moamba	MZ3	20	25°55'S	32°55'E	0-200
Namibia					
Oshikondilingo	N1	16	*	*	*
OhangweNa	N2	15	*	*	*
Kaimbedza	N3	20	*	*	*
Zambia			*	*	*
Zambia	ZA2	16	*	*	*
Zambia	ZA3	14	*	*	*
Zimbabwe					
Ngundu	Z1	13	20°50'S	32°50'E	*
Mudzi	Z2	15	16°17'S	32°45'E	400
Biriwiri	Z3	15	19°50'S	32°40'E	1 500
Mzarabani	Z4	15	19°50'S	32°40'E	600
Matebeleland N	Z5	14	18°00'S	29°00'E	Na
Matebeleland S	Z6	15	21°00'S	32°45'E	Na
Swaziland	S 1	20	26°45'S	31°45'E	Na
Tanzania					
Makadaga (Mbarali)	T1	Р	8°40'S	34°35'E	1 200
Nyamahanga (Iringa)	T2	Р	7°49'S	35°52'E	950
Mkata (Kilosa)	Т3	Р	7°22'S	37°50'E	430
UbeNa (Bagamoyo)	Τ4	Р	6°11'S	38°10'E	305
Chalinze (Bagamoyo)	Τ5	Р	6°55'S	38°20'E	550
Magamba (Turiani)	Т6	9	5°40'S	38°12'E	530
Kigwe(Dodoma)	Τ7	Р	6°04'S	35°45'E	920
Mialo (Kondoa)	Т8	Р	5°03'S	36°21'E	1 150
Mandimu (Singida)	Т9	Р	5°04'S	35°08'E	1 460
Kigwa (Tabora)	T10	Р	5°35'S	33°32'E	1 200
Mali					
Missira	M1	19	13°43'N	8°27'W	352

 Table 1. Seed sources of Sclerocarya birrea: A provenance trial planted in Malawi.

Source: Chirwa et al. (2007).

Magunde provenances showed significantly higher aphid infestation than all other provenances (Table 6). Mzarabani showed significantly least infestation of aphids than all provenances in both of the two seasons.

Screening provenances of disease prevalence

Provenances were screened on disease prevalence and showed significant differences in cankers, dieback and

 Table 2. Key for assessment score of insect pest damage on foliage.

Scale	Rate (%)	Damage
1	0-10	No damage
2	11-25	slight damage
3	26-50	Extensively damaged
4	51-75	Severely damaged
5	76-100	Completely damaged

Source: Adopted from Burks and Brandl (2005).

Table 3. Key for aphid infestation rating scale.

Rating	Incidence	Infestation/severity		
1	0	No infestation		
2	≤ 100	Slightly infested		
3	≥ 200 but ≤ 300	Moderate		
4	≥300 but ≤ 400	High infestation		
5	≥ 400	Severe infestation		

Source: Adopted from Litsinger et al. (1998).

 Table 4. Disease assessment rating scale.

Scale	Scale (%)	Susceptibility	
1	1-10	Resistant/tolerant	
2	11-30	Moderately resistant	
3	31-60	Moderately susceptible	
4	61-100	Susceptible	

Source: Adopted from MaNandhar et al. (2016).

leaf lesions ($P \le 0.001$) (Figure 1). Chikwawa, Mangochi, Mzarabani and Ngundu were significantly higher occurrence of cankers. Kaimbedza, Mzarabani, Mangochi and Chikwawa showed higher prevalence of die back as opposed to Ngundu, Ntcheu and Swaziland which were highly attacked by leaf lesions (Figures 2 and 3).

DISCUSSION

The study results indicated significant differences in the infestation and damage of insect pests between provenances of *S. birrea*. The variations in the infestation and damage by of insect pests in *S. birrea* provenances indicate how a selected genotype is likely to be susceptible to insect pests. The provenances including Magunde, Mangochi, Chikwawa, Marracuene and Rumphi showed high susceptibility scores in regards to defoliators, skeletonizers and aphid (Table 6). These

 Table 5. Scale on estimating lesion on infected leaves.

Observation	Scale (%)	Response		
No lesion	1-10	Resistant		
0.1-2cm	11-30	Moderately resistant		
2.1-4cm	31-60	Moderately susceptible		
>4cm	61-100	Susceptible		

Source: Adopted from Karan babu et al. (2013).

provenances are early flushers and suffered a serious infestation contrary the result of Msukwa et al. (2019).

Earlier studies by Msukwa et al. (2019) and Reddy (2013) asserted that trees species which flush early in the dry season suffer significant lower damage of herbivory insects than of late leaf flushers, however this has revealed that Magunde and Marracuene provenances though early leaf flushers, suffered significantly high herbivory insect pest infestations. In this regard it agrees with Loney et al. (2006) that early leaf flushers expose tender and juicy leaves which are favourable to insect pests due to its palatability since Magunde and Marracuene had tender leaves that can probably attract insect pests to feeding.

This study has further revealed that Moamba provenance regardless of being early leaf flushers showed some levels of resistance to insect pest pressure (Table 6). This provenance might have some leaf mechanical traits which this study did not validate, however, He et al. (2019) suggested that such plants contain such traits to resist pests damage. Though not statistically proven, the Moamba provenance might have had this ability to deter insects feeding behavior as it had significantly lower damage scores than others. Subsequently, suggestions made by Clissold et al. (2009) provide possibility of such plants having phenotypic and genetic defensive mechanisms deterring insect feeding activities. The possibility of S. birrea provenances could be a function of genetic arrangement of the materials in question. Therefore, the significantly lower damage observed in provenances such as Moamba could partly be attributed to its genetic arrangement with an interaction of the environment. However, more work should be done to ascertain these observations.

Infestations of provenances to defoliators was to be understood on its complexly in the manner that defoliation in plant is a usual phenomenon. For instance, He et al. (2019) indicates that all woody plants periodically shed their leaves despite multiple contributing factors. In similar manner, Robert *et al.* (2002) suggests that leaf shedding is regulated by hormones in response to environmental stress factors such as pest attack, water stress and photoperiod. During this study, visual observations revealed that high probability of leaf defoliation was more of an effect of insect pest infestation

Provenance —	Mean infestation score (defoliators)		Mean infestation score (skeletonizers)		Mean infestation score (aphid)	
	2018/19 Season	2019/20 Season	2018/19 Season	2019/20 Season	2018/19 season	2019/20 Season
Mzarabani	1.063 ^a	1.062 ^a	2.65 ^{efg}	3.175 ^{ghi}	1 ^a	1 ^a
Oshikondilingo	1.238 ^{ab}	1.237 ^{ab}	2.35 ^{ab}	2.825 ^{efg}	1.175 ^{abc}	1.175 ^{abc}
Zambia2	1.312 ^{abc}	1.263 ^{abc}	2.425 ^{ab}	2.313 ^{cde}	1.175 ^{abc}	1.25 ^{abcd}
Changwena	1.5 ^{bcd}	3.163 ^k	2.575 ^{bc}	2.088 ^{bcd}	1.337 ^{cde}	1.65 ^{fgh}
MatebelelandiN	1.512 ^{bcd}	1.513 ^{bcd}	2.413 ^{ab}	2.55 ^{def}	1.300 ^{bcd}	1.325 ^{cde}
Mudzi	1.537 ^{bcd}	1.8 ^{def}	2.45 ^{ab}	2.45 ^{def}	1.587 ^{efg}	1.662 ^{fgh}
Swaziland	1.625 ^{cde}	1.625 ^{cde}	2.363 ^{ab}	1.85 ^{abc}	1.2 ^{abc}	1.2 ^{abc}
Zambia	1.75 ^{def}	1.75 ^{def}	2.413 ^{ab}	2.113 ^{bcd}	1.05 ^{ab}	1.05 ^{ab}
Biriwiri	1.913 ^{efg}	1.8 ^{def}	2.587 ^{bc}	1.45 ^a	1.512 ^{def}	1.725 ^{gh}
Ngundu	1.938 ^{efg}	1.938 ^{efgh}	2.65 ^{de}	2.963 ^{fgh}	1.325 ^{cd}	1.325 ^{cde}
Mali	1.962 ^{efg}	1.838 ^{defg}	2.65 ^{cde}	2.925 ^{fgh}	1.362 ^{cde}	1.5 ^{defg}
Magamba	2.013 ^{fg}	2.013 ^{fghi}	2.65 ^{de}	2.95 ^{fgh}	1.812 ^{gh}	1.625 ^{fgh}
Kaimbedza	2.175 ^{gh}	2.3 ^{hi} j	2.188 ^a	2.063 ^{bcd}	1.837 ^{gh}	2.112j
Ntcheu	2.213 ^{gh}	2.213 ^{ghi} j	2.55 ^b	2.313 ^{cde}	1.412 ^{cde}	1.412 ^{cdef}
MatebelelandiS	2.388 ^h	2.388 ⁱ j	2.625 ^{bcd}	2.675 ^{efg}	1.762 ^{fgh}	1.675 ^{gh}
Moamba	2.388 ^h	2.3 ^{hi} j	2.65 ^{ef}	3.725j	1.5 ^{de}	1.512 ^{efg}
Rumphi	3.088 ⁱ	3.088 ^k	2.65 ^{fgh}	2.9 ^{fgh}	2.688 ⁱ	2.687 ^k
Marracuene	3.2 ⁱ	3.2 ^k	2.65 ^{hi}	3.625 ⁱ j	1.862 ^h	1.725 ^{gh}
Mangochi	3.238 ⁱ	3.238 ^k	2.65 ^{bcd}	1.65 ^{ab}	2.562 ⁱ	1.987 ⁱ j
Chikwawa	3.238 ⁱ	2.525j	2.65 ^{ghi}	2.313 ^{cde}	2.662 ⁱ	2.437 ^k
Magunde	3.338 ⁱ	3.262 ^k	2.65 ⁱ	3.3 ^{hi} j	1.912 ^h	1.812 ^{hi}
LSD	0.3625	0.3815	0.3472	0.5489	0.2564	0.2562
CV %	55	54.9	40	49.7	51	51.3

Table 6. Infestation mean score for defoliators, skeletonizers and aphids.

Means followed by the same letter are not significantly different according to Fisher's least significant difference test. Source: Authors

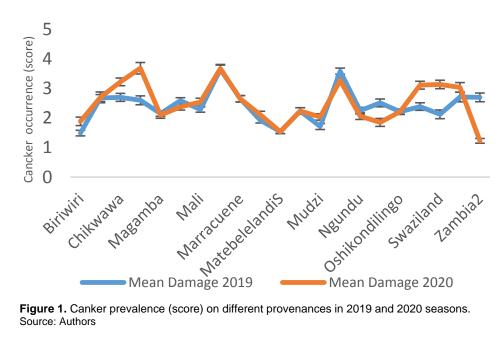


Figure 1. Canker prevalence (score) on different provenances in 2019 and 2020 seasons. Source: Authors

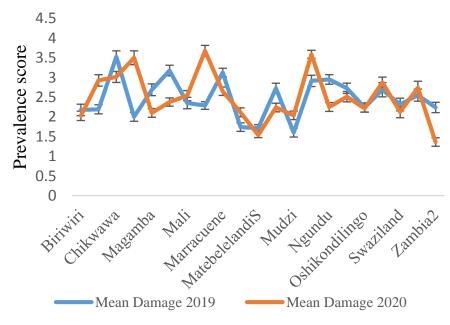


Figure 2. Screening of provenances to dieback in 2019 and 2020 seasons. Source: Authors

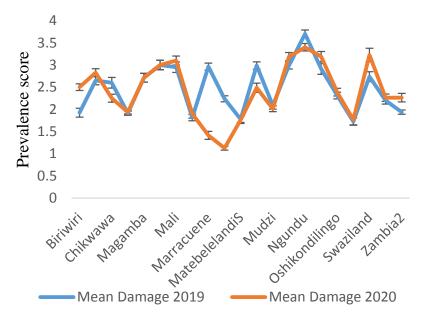


Figure 3. Screening on leaf lesions prevalence 2019 and 2020 seasons. Source: Authors

than periodic behavior of plants (Robert *et al.*, (2002). However, the process of identifying provenance resistance to insect pests requires understanding of the interaction between the said provenances and the hostinsect (Sniezko and Koch, 2017). It is also important to further understand the phenology of the *S. birrea* based on host-insect interactions which is so dependent on phenotypic traits. This however could be explained if the host-insect interactions were understood within the insect life cycle of the host plant. In the case of Magunde, the level of recovery was slow and much of the twigs were dying off. Smith, (2005) argued that the plant may interact negatively with insect pest and quickly recover and less harm occurs and such a plant shows the high level of tolerance.

Cankers, die back and leaf lesions were prominent in all provenances. However, Kaimbedza, Mangochi and Mzarabani significantly showed high levels of canker infestation which were basically due to physical injury. Mangochi and Chikwawa were physically wounded as were said to be sources of medicines. This action led into an increase in provenances cankers attack. Despite of the cankers occurrence were due to physical stress; the fungal infections were noted as secondary infections taking advantage of the openings. Wegulo (2011) reported that most common fungal diseases are related to adverse environmental stress. This therefore supports the findings that the provenances which were susceptible to cankers were due to stress induced by pests.

However, in addition to canker disease, studies have indicated that trunk damaging fungi largely cause serious disorders as dieback, vascular wilt and wood rot diseases. In this study, it was observed that Mzarabani, Mgunda, Ntcheu and Swaziland were oozing especially on fresh injury (Gonthier et al., 2015) and Parker (1978) suggest that such damage contributes to dieback. Despite lack of evidence in this study there is a wide understanding that *Botryosphaeria* species may have contributed to die-back and canker diseases on woody hosts (Gonthier et al., 2015). This is because it has a wider host range as reported to attack over 100 species including apple, eucalypts, grevillea, guava, loquat, macadamia, mango, maple, mulberry, papaya, peach and pear (Sinclair and Byrom, 2006) so is *S. birrea*.

CONCLUSION AND RECOMMENDATIONS

The screening process of the provenances has revealed that the level of susceptibility and tolerance varies between provenances. Some provenances were highly susceptible to pests and diseases than others. The study has further revealed that insect pressure especially defoliators and skeletonizers more prominent is Sclerocarya birrea. This therefore suggests that the selected genotypes for domestication require more effort on pest management. The study has revealed diseases of economic importance such die back, leaf lesions and cankers are to be considered as an advancements in S. birrea to domestication. Therefore, when promoting the.0000.se provenances for domestication, pests and diseases management should considered as an important selection tool. There is need to propagate and safeguard promising elite provenances for potential genotypes.

Data availability

The data used to support the findings of this study are

available from the corresponding author upon request.

Disclosure

The authors take full responsibility of any error. The content of this document is a sole responsibility of the author and can under no circumstances be regarded as reflecting the position of the institutions support to this work.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest to publication of this research work

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