

African Journal of Plant Science

Full Length Research Paper

# Infestation dynamics of mistletoes in urban and rural landscapes of semi-arid Botswana

Mpho Granny Batlhophi<sup>1\*</sup>, Tebogo Selebatso<sup>1</sup>, Keoikantse Sianga<sup>2</sup>, Mpho Rinah Setlalekgomo<sup>1</sup>, Boipuso Legwatagwata<sup>1</sup>, Ross N. Cuthbert<sup>3</sup> and Mmabaledi Buxton<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

<sup>2</sup>Department of Wildlife and Aquatic Resources, Botswana University of Agriculture and Natural Resources, Private bag 0027, Gaborone, Botswana.

<sup>3</sup>School of Biological Sciences, Queens University Belfast, Belfast, BT9 5DL, United Kingdom.

Received 14 March, 2024; Accepted 15 May, 2024

The available information regarding mistletoes inadequately explains the dynamic facets of infestation in rural-urban gradients. This study was carried out to compare the abundance of mistletoe species on hosts between urban (Gaborone and Gakuto) and rural (Lentsweletau and Oodi) areas of Botswana. Four plots each with a surface area of 625 m<sup>2</sup> were established in the respective study areas and from which mistletoe-bearing hosts were examined. Ten indigenous host plant species from six families were found infested with four parasitic plants, *Erianthemum virescens, Plicosepalus kalachariensis, Viscum rotundifolium,* and *Viscum verrucosum.* Urban areas had more infested hosts, with *P. kalachariensis* dominating. *V. rotundifolium* parasitic plant-associated infections were primarily rural-based. More infestation was skewed toward *Vachellia* host species. Mistletoes from two different families could parasitise a single host. Pairwise comparison tests suggested significant differences in hosts infested by *E. virescens* relative to *V. verrucosum, V. rotundifolium,* and *P. kalachariensis,* respectively. The findings provide baseline information documenting parasitism in host species in varied landscapes. Further studies may explore latching mechanisms across host species, bio-physical parameters that enhance infestation, parasite-host diversity, and distribution owing to the infestation potential of the species.

Key words: Host, indigenous plants, land use, mistletoe-infections, parasitism.

# INTRODUCTION

Parasitic flower-bearing plants, vernacularly named *boswa* in Botswana (Setshogo, 2005), constitute a group of plants that exploit other plants as animate hosts for growth and survival (Těšitel, 2016). The word *boswa* is derived from the statement "*go swa*" which means "to die". It implies that the host plant would possibly die from

parasitism (Setshogo, 1998). The distinctive feature of a parasitic plant is the establishment of a haustorium, an organised morphological machinery that links the parasite to the host's vascular vessels (Bouwmeester et al., 2021; Teixeira-Costa and Davis, 2021). The haustorium apportions the materials in the vascular tissues of the

\*Corresponding author. E-mail: <u>mbatlhophi@buan.ac.bw</u>. Tel: +2673650562.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> host to itself (Twyford, 2018). Parasitic plants are categorised as hemi or holo, respectively, based on the ability or inability to photosynthesise. Groupings may also depend on the parasite's site of attachment (trunk or root) on the host. Furthermore, they are classified as facultative or obligate; the former can end their life cycle without the host's contribution, contrary to the latter (Yoshida et al., 2016). Since holoparasites are devoid of photosynthetic functionalities, they depend on the host for all growth essentials (Casadesús and Munné-Bosch, 2021). Conversely, hemiparasites photosynthesise to ensure their carbon supply. The photosynthetic machinery enables them to course through rather thick hosts' rhytidomes (Těšitel, 2016).

Parasites with obligate host stem-dependent life cycles, as well as self-sourced carbohydrates, are called aerial hemiparasites or mistletoes (Wahyuningtias et al., 2021; Muche et al., 2022). Mistletoes belong to the order Santalales (sandalwood), comprising five families as Eremolepidaceae, Loranthaceae, Misodendronaceae, Santalaceae, and Viscaceae (Wahyuningtias et al., 2021) of which three, Santalaceae, Loranthaceae and Misodendronaceae comprise hemiparasites (Niu et al., 2024). Most mistletoes belong to Loranthaceae (Gairola et al., 2013). These parasites are implicated in causing harm of varying degrees to the hosts and are a menace to agricultural production in less developed countries (Bouwmeester et al., 2021) as they attack crops and fruit trees (Watson et al., 2020; Erdogan, 2022; Musselman and Rodenburg, 2023). Apart from the host's ascent of sap being used as a source of water and minerals, the epiphytic mistletoes settle on the host's trunk positions to maximise access to light for photosynthesis (Teixeira-Costa and Davis, 2021). Though a larger portion of the mistletoes' carbon is self-generated, the parasites can tap slightly into the host's phloem reservoir of organic nutrients (Muche et al., 2022) to compensate for their lower carbon yield than their host's (Griebel et al., 2017). Xylem-drawing parasitic plants are the most predominant in the southernmost parts of Africa with semi-arid climates (Veste, 2007). Several woody species can serve as hosts for more than one parasitic plant (Těšitel et al., 2021). Similarly, mistletoes may exhibit host plant compatibility with a variety of hardwoods and plants of a shrubby form (Gairola et al., 2013; Ma et al., 2020). Some of the parasites have a generalist approach as they infect many dissimilar host species. Others concentrate on parasitizing a limited number, for instance singling out a particular host for infestation (Walker et al., 2017). Host plants in disturbed, urban abodes are photophilous (Fontúrbel et al., 2017), and are subjected to harsh ambient conditions, therefore they tend to be more easily parasitized than those in rural areas (Skrypnik et al., 2020; Niu et al., 2024). Infestation of the hosts by mistletoes depends on the choice that seeddispersing birds make as to which trees they prefer as roosts (Muche et al., 2022). Since birds alight deliberately

on trees, the seeds of mistletoes are unequally distributed among host trees (Okubamichael et al., 2016). Not all the seeds defaecated on trees germinate. The tree must be vulnerable to infection by the mistletoe. Thus, the most plenteous host trees are more prone to infection provided regular visitation by birds and susceptibility to mistletoe-infection conditions are met. Trees that do not meet these requirements are highly disposed of for zero infection by mistletoes, even if they are available within a habitat (Madisa et al., 2017). Landscape heterogeneity elicits differential host plant infestation by mistletoes (Magrach et al., 2015). The diversity and distribution of mistletoes are facilitated by several factors including inter alia parasitic plant's choice and compatibility (e.g., immunity), dynamics of seed dispersal agents (e.g., wind, birds), and abiotic parameters (Niu et al., 2024). For instance, parasitism may be influenced by the physiochemical attributes of host plants, ambient environmental conditions, and the ability of the parasitic plant to overcome the host's defence response against exploitation (Muche et al., 2022).

Diverse habitats in Botswana are prone to infestation by parasitic plants, yet studies on these plants are rare. However, Madisa et al. (2017) identified four mistletoe species in an urban area, namely Erianthemum ngamicum, Plicosepalus kalachariensis, Tapinanthus oleifolius, and Viscum album. The latter belongs to Viscaceae (Valle et al., 2021) while the former three are Loranthaceae members (Grímsson et al., 2018), These were found on the host plants; Vachellia eriobola, Senegalia mellifera, Terminalia Ziziphus sericea, mucronata. Schinus molle. Vachellia tortilis. Senegalia erubescens, and Vachellia flekii. This highlighted insufficiency in the documentation of parasitic-host species diversity and distribution as the study focused on a specific urban location. The current study was aimed at exploring the diversity and colonisation of mistletoes and familiar host plants in stratified landscapes. Therefore, we hypothesised that: (i) urban areas are likely colonised by mistletoes more than rural areas due to the variety of host species associated with urbanisation; (ii) certain host species may bear more parasitic plants than others following dynamics of host infectivity, and that; (iii) there is significant difference between species of mistletoes when the host is parasitised multiple times. The findings of this study may not only document parasitic and host plant species inventory databases, but also inform environmental policymakers to formulate restorative and managerial approaches against deformed habitats in Botswana.

## MATERIALS AND METHODS

#### Study areas

The study was carried out in two urban areas represented by



Figure 1. Location of Botswana in Africa a) showing sampled Districts b) (Kweneng, South-East and Kgatleng) across urban (Gaborone and Gakuto) and rural (Lentsweletau and Oodi) gradient.

Gaborone and Gakuto, in Southeast and Kweneng districts, respectively, and two rural habitats: Lentsweletau and Oodi, located in Kweneng and Kgatleng districts, respectively (Figure 1). Gaborone site 1 (urban) was situated in a bushy patch of land, adjacent to the shopping areas; while site 2 was near a residential zone. Both sampling sites were located near busy tarred roads. Sites sampled in Gakuto were situated on land closer to sites previously used for agricultural purposes. However, farming at this site has ceased to allow for Gaborone-city expansion (Mosha, 2016). Most of the land around the sampling sites was predominantly undergoing residential development. Oodi's sampled sites were rocky, with Fabaceae species dominating the area (Tshireletso et al., 2018). The sampling sites were 1 km away from residential areas with evidence of minimal ploughing activities. The area of Lentsweletau is characterised by grassy woodland (Tshireletso et al., 2018). Sampling was done 1 km from the tarred road, within areas constituted by free-range farming practices. On average, Gaborone rainfall amounts to 450-550 mm per year (Arsiso and Mengistu Tsidu, 2023), while Kweneng and Kgatleng regions experience precipitation amounting to between 350 and 600 mm, annually (Makhabu et al., 2021). The rainfall is mainly experienced around October and subsides by the end of April (Botswana, 2017).

## **Data collection**

The data assessment was done between September and November 2023. The choice for the areas of study was based on the observation of host trees infested with mistletoes across the localities. Each sampled site measured 50 m away from the main road. The major limitation on the sample size was the availability of land due to factories, residential houses, and ploughing fields around the sites in urban and rural areas respectively. A site was demarcated into two transects distanced 10 m apart. Each transect was divided into four plots measuring 25 m × 25 m (625 m<sup>2</sup>), each. Both exotic and indigenous host species were assessed for mistletoe infestation. All host plants with associated mistletoe

assemblages were examined, identified, and counted in each established site across urban-rural spheres. Host trees infested with one and/or two parasitic plant species were recorded separately. The mistletoes and host trees were identified morphologically to species level on-site following protocols of Van (2013). The number of parasitic plant(s) infesting the hosts was recorded per mistletoe species in both single and multiple-species infestations. In addition, un-infested tree and shrub species within the sampled sites were identified, counted, and recorded.

#### Data analysis

A generalized linear model was used to determine whether there was a relationship between sites (urban and rural), host and parasitic plants. An independent paired t-test was used to determine significant differences between the number of host plants in urban and rural areas, infested plants in urban and rural areas, and uninfested host plants in urban and rural areas in R (RCore-Team, 2013). To test a hypothesis that a single host plant was infested by multiple parasitic plant species, data sets of hosts with more than one parasitic plant were tested for the assumptions of linear covariation and normality in R (RCore-Team, 2013). After meeting the assumptions of normality, the data was subjected to the Pearson Correlation test in R (RCore-Team, 2013).

## RESULTS

Examples of mistletoe species found on some indigenous host plants in Gaborone and Gakuto are presented in Figure 2; and a list of host trees parasitised by mistletoe is presented in Table 1.

The results suggested that there were no significant differences in the number of hosts, and uninfested plant species between urban and rural areas, respectively (t = -



**Figure 2.** Mistletoe species identified on some indigenous host plants in Gaborone and Gakuto. **A**, *Erianthemum virescens* on *Vachellia erubescens*; **B**, *Plicosepalus kalachariensis* on *Vachellia tortilis*; **C**, *Viscum verrucosum* on *Vachellia luederitzii* and **D**, *Viscum rotundifolium* on *Ziziphus mucronata*.

Host tree species	Family		
Ziziphus mucronata	Rhamnaceae		
Vachellia tortilis	Fabaceae		
Senegalia erubescens	Fabaceae		
Boscia albitrunca	Capparaceae		
Senegalia mellifera	Fabacaeae		
Vachellia nilotica	Fabaceae		
Vachellia luederitzii	Fabaceae		
Ehretia rigida	Boraginaceae		
Grewia flava	Tiliaceae		
Maytenus senegalensis	Celastraceae		

Table 1. Host tree species found parasitised in urban and rural areas.

0.224, df = 2, p-value = 0.825; t =1.011, df = 2, p-value = 0.419) (Figure 2). Host plants in urban areas were more infested by parasitic plants relative to rural areas (t = 1.022, df = 2, p-value = 0.0417) (Figure 3).

The results also suggested no significant difference between the number of infested tree genera in urban and rural areas (t = 0.986, df = 2, p-value = 0.362) (Figure 4).

The genus *Vachellia* was mostly infested in urban areas compared to rural areas (Figure 4).

There was a correlation between host and parasitic plants across sites (t = 3.47, df = 103, p-value = 0.0008). In urban areas, *Z. mucronata* was associated with *Viscum verrucosum*, *Viscum rotundifolium*, *P. kalachariensis*, and *Eigenmannia virescens* parasitic plants; whereas, the











# Infested genus in urban and rural areas

**Figure 4**. Infested tree genera in urban and rural areas (*ZIZ* = *Ziziphus*, *VAC* = *Vachellia*, *SEN* = *Senegalia*, *BOS* = *Boscia*, *GRE* = *Grewia*, *COM* = *Combretum* and *MAY* = *Maytenus*.



**Figure 5.** Hosts and their associated parasitic plants in urban and rural areas (*ZIZMUC* = *Z. mucronata*, *VACTOR* = *V. tortilis*, *SENER* = *S. erubescens*, *BOSALB* = *B. albitrunca*, *SENMEL* = *S. mellifera*, *VACNIL* = *V. nilotica*, *VACLUE* = *V. luederitzii*, *EHRRIG* = *E. rigida*, *GREFLA* = *G. flava* and *MAYSEN* = *M. senegalensis*).

SENRE (S. erubescens) and BOSALB (Boscia albitrunca) were associated with V. rotundifolium, P. kalachariensis and E. virescens parasitic plants (Figure 5). SENMEL (S. mellifera) and VACLUE (Vachellia luederitzii) were associated with V. verrucosum and P. kalachariensis, respectively (Figure 4). In rural areas, BOSALB (B. albitrunca) was associated with V. verrucosum, V. rotundifolium and P. kalachariensis, and E. virescens parasitic plants (Figure 5). VACTOR (V. tortilis), SENER (S. erubescens), GREFLA (Grewia flava) and VACNIL (Vachellia nilotica) were associated with V. verrucosum, V. rotundifolium, V. rotundifolium and E. virescens parasitic plants, respectively (Figure 5).

Parasitic plants (VV= V. verrucosum, VR = V. rotundifolium, ER= E. virescens, PK = P. kalachariensis) of different species parasitise a single host tree (VACTOR = V. tortilis, SENMEL = S. mellifera, VACNIL = V. nilotica, VACLUE = V. luederitzii and GREFLA = G. flava) at the same time.

The results suggested a significant correlation between hosts and parasitic plants across sites (t

= 3.4667, df = 103, p-value = 0.0008). In urban and rural areas, pairwise comparison tests suggested significant differences in host plants infested by *E. virescens* relative to *V. verrucosum*, *V. rotundifolium*, and *P. kalachariensis*, respectively (Mean Difference = 2.01, 2.12, 0.799; Mean Standard error= 0.367, 0.655, 0.326; p-value = 0.00, 0.002, 0.016) (Table 2). Similarly, *P. kalachariensis*-

infested hosts were significantly different from those infested by *V. verrucosum, V. rotundifolium,* and *E. virescens,* respectively (Table 2). However, the hosts infested by *V. verrucosum* were not significantly different from those infested by *V. rotundifolium* (Mean Difference = 0.108; Mean Standard error = 0.659; p-value = 0.870) (Table 2). The results also demonstrated that *V. verrucosum* and *V. rotundifolium* cohabitated the *V. tortilis, V. luederitzii,* and *S. mellifera* in urban and rural areas but were not observed as parasites on *G. flava;* whereas, *E. virescens* and *P. kalachariensis* were observed as parasites on *G. flava* (Table 3). *V. verrucosum, V. rotundifolium, E. virescens* and *P. kalachariensis* were observed to be parasites of the host *S. mellifera* (Table 3).

## DISCUSSION

The results of this study showed relatively similar amounts of hosts and non-infected plant species in urban and rural areas of Botswana. None of the host trees identified were exotic or dead, except for a few cases where one or two branches of the hosts had dried up together with the parasitic plant infesting them. The mistletoes found in both urban and rural landscapes were the Loranthaceae, *E. virescens*, *P. kalachariensis*, and the Viscaceae members represented by *V. verrucosum* 

Parasite		Mean difference	Mean. Std. Error	P value
	V. rotundifolium	0.108	0.659	0.870
V. verrucosum	E. virescens	-2.014*	0.367	0.000
	P. kalachariensis	-1.215*	0.334	0.000
	V. verrucosum	-0.108	0.659	0.870
V. rotundifolium	E. virescens	-2.121*	0.655	0.002
	P. kalachariensis	-1.323*	0.637	0.040
	V. verrucosum	2.014*	0.367	0.000
E. virescens	V. rotundifolium	2.121*	0.655	0.002
	P. kalachariensis	0.799*	0.326	0.016
	V. verrucosum	1.215*	0.334	0.000
P. kalachariensis	V. rotundifolium	1.323*	0.637	0.040
	E. virescens	-0.799*	0.326	0.016

Table 2. Pairwise comparisons of parasitic infestation across host plants in urban and rural areas.

**Table 3**. Cohabitation of parasitic plants in urban and rural areas.

Parasite	Hosts					Tatal	
	Vactor	Vacnil	Vaclue	Seneru	Senmel	Grefla	Total
VV	11	3	6	5	1	0	26
VR	3	0	1	0	1	0	5
EV	0	2	0	14	11	1	28
PK	9	2	5	14	13	1	44

and *V. rotundifolium.* Mistletoe parasitism was mostly prevalent in urban areas as opposed to rural environments. The reason is that most urban sites constitute landscaped areas, bordered by trees of the same species, thus increasing the accessibility of the trees for parasitism by mistletoes (Niu et al., 2024). According to Skrypnik et al. (2020), urban trees are also subjected to more challenging conditions and thus vulnerable to parasites, overall. The investigation revealed a total of seven infested genera with no significant difference between the urban landscape and the rural countryside. The most abundant and highly infested host trees belonged to the Fabaceae.

Whilst Dzerefos et al. (2003) describe cases in which host preference by mistletoes was indirectly proportional to host species density; this was not observed in either of the sampled landscapes. Rather, the mistletoes infested the most abundant host species in the area. This conforms with Dlama et al. (2016) and Tizhe et al. (2020), who both established the highest figures in mistletoe infection in two distant landscapes among predominant Fabaceae species; namely, *Albizia lebbeck* and *Vachellia indica*, respectively. Studies carried out by Dean et al. (1994) and Wahyuningtias et al. (2021) also revealed Fabaceae as the family having the highest level of infection. Members of the Fabaceae have been established as one of the most available and susceptible to infection by mistletoes in Africa (Krasylenko et al., 2022). The mistletoe seed traits render them prone to wind dispersal and thus easily spread out in various environments (Tizhe et al., 2020). Moreover, Fabaceae members are tree legumes recognised as being rich in nitrogen forms, thus supporting growth of more mistletoes (Matthies, 2021). Amongst the infested genera, mistletoe infestation was prevalent among Vachellia host trees in urban territories. In congruence, Al-Robai (2023) and Madisa et al. (2017) also recorded the highest number of on Acacia host trees on mistletoes separate investigations. The researchers described Acacia (currently named Vachellia) members as suitable for mistletoe infection based on their large canopies. Vachellia species are normally prolific in semi-arid grassland ecosystems, occupying an indispensable role in the communal economic status of humans. The obvious occurrence of these species could be ascribed to their ability to grow competitively under harsh conditions

(Karlin and Karlin, 2018).

In the present study, a relatively low number of host tree species (10) were found infested with the parasitic plants. The four mistletoes were found on all the host species so they might be regarded as generalists as opposed to specialists. This was expected, since mistletoes with a wide spectrum of host species acquire more resources than those which particularise on hosts (Tizhe et al., 2020). As per the study by Godschalk (1983), the mistletoes V. rontundifolium and *V*. *verrucosum* are specialists. A preceding study by Okubamichael et al. (2011) identified V. rotundifolium concisely infesting Z. mucronata and B. albitrunca in South Africa and Namibia, respectively. However, in our study the two mistletoes infested different hosts as did P. kalachariensis and E. virescens. This resonates well with the finding in which V. rotundifolium was found parasitising six trees (Okubamichael et al., 2016). Surprisinaly. Vachellia nilotica which seemed predisposed to infestation by three parasites (E. virescens, V. rotundifolium, and V. verrucosum) in urban sites, turned out infected by only E. virescens in rural areas. It is still entirely possible that V. nilotica is liable to infection by V. rotundifolium and V. verrucosum but manages to "escape" the mistletoes in a different site due to other factors such as behaviour of seed dispersers, and the presence of better hosts. Again, this erratic choice of hosts affirms the view by Okubamichael et al. (2016), that a parasitic plant can shift between being a generalist to a specialist under different biotic and abiotic prompts. Besides, host-specific mistletoes are unlikely to occur in habitats with hosts existing in low abundance (Norton and Carpenter, 1998; Hishe and Abraha, 2013). Perhaps for this reason, the four mistletoes shared hosts, albeit with varying degrees of infestation. In this study, we did not investigate abiotic parameters that could modulate this shift. Therefore, future studies may unravel the dynamics of bio-physical parameters that facilitate parasite infestation.

Each mistletoe species infected a minimum of 50% of the host tree species in urban areas as opposed to 25% in rural environments. The host trees in urban and rural settings differed in their association with the four parasitic plants. The parasites P. kalachariensis, E. virescens, and V. rotundifolium were the most common in urban and rural areas, respectively. While each of the three parasites infested at least 75% of the host species, the infestation caused by P. kalachariensis parasite was more prominent than its counterparts (E. virescens and V. rotundifolium), especially on two closely linked species, S. erubescens and S. mellifera. The note that the latter two hosts are exploited by the same parasitic plant species comes not as a surprise. It is an affirmation of the explanation given by Norton and Carpenter (1998) that hosts confined to a single taxonomic genus category or evolutionary line can be infected by the same parasite.

In instances where a host was infected by multiple

parasitic species, the four parasites assumed a clear pattern. For urban, Loranthaceae mistletoes (P. kalachariensis and E. virescens), showed a degree of infestation commonality among hosts, namely Z. mucronata, S. erubescens, B. albitrunca, and E. rigida belonging to four families. The sum of the mistletoes per host tree was the same except for those found in S. erubescens where P. kalachariensis counts were higher than for E. virescens. A similar trend appeared in rural areas where Viscaceae mistletoes (V. rotundifolium and V. verrucosum), explored B. albitrunca, V. luederitzii and *M.* senegalensis from three families. Here, the notion that parasitic plants of close descents scout around for the same hosts (Matthies, 2021) is supported but it is not in conformity with the view by Norton and Carpenter (1998), that the hosts are of the same family. Overall, preference for specific hosts by both Loranthaceae and Viscaceae mistletoes was low. Rather, infection was more pronounced in larger and high-standing trees just as they have been described as the most preferred by birds. Perfectly elucidated, the dissimilarities observed in the fruit structure of Viscum mistletoes determine the kind of birds that disperse them (Godschalk, 1983). The reasons behind loranthoid and sandalwood species scavenging better for hosts in urban and rural areas, respectively, are beyond the scope of this study.

It was inferred that the four mistletoes were associated with the host trees *Z. mucronata* and *Boscia albitrunca* in urban and rural areas, respectively. The results show congruence with the research carried out by Madisa et al. (2017) in one urban site, documenting *Z. mucronata* and *Acacia erioloba* (now *Vachellia erioloba*) as the bestchosen hosts for infestation. Since the fruits of *Z. mucronata* and *B. albitrunca* are edible (Motlhanka et al., 2008) and thus the best-loved by frugivorous birds (Alias et al., 2003; Mokgolodi et al., 2011), they could be frequently visited for feeding thus having more mistletoe seeds left on them by the birds.

Concerning cohabitation of the parasitic plants, a significant number of hosts were concomitantly infested by two distinct mistletoes. Parasitic plants belonging to different families could synchronously parasitise the same host except for G. flava which tended prone to infection by the loranthoid species, E. virescens and P. kalachariensis. Notably, G. flava occurred only in rural areas and was infested by none except the parasite V. rotundifolium under one-to-one parasite-host relations. The parasites, P. kalachariensis and V. rotundifolium were respectively the most and least frequently encountered in coexistence within urban and rural localities. Meanwhile, the parasite V. rotundifolium was rarely found in the share-host relations. Intriguingly, in those cohabitation occurrences, the abundance of the parasites was significantly different. The parasitic plants grew independently, appreciably interspersed on the stem of the host, and appeared smaller in size and lower in number compared to when growing alone on a single

host. To the best of our knowledge, this study is the first to establish cohabitation behaviour regarding specific mistletoes in Botswana. The factors impacting the choice of a single host by multiple mistletoes are not known and exceed the scope of this study.

Although every sampled landscape had host trees, not all were parasitised. For instance, occurrence of mistletoes was consistently nil for Peltophorum africanum, and Boscia foetida despite them being large in size and plentiful in all the sites. This phenomenon is predictable, because an inquiry into the extensiveness of mistletoe infection in an Acacia-dominated area of central Africa recorded non-mistletoe-bearing hosts (Roxburg and Nicolson, 2005). Probably, P. africanum and B. foetida are either rarely opted for rest by mistletoe seeddispersing birds, or are highly resistant to parasitism by the mistletoes (Tizhe et al., 2020). This was underscored by the large number of nests on the most invaded host plants: for instance. Vachellia species as opposed to the trees devoid of bird's nests where no mistletoe infestation was observed. Since humans are increasing in population, more habitats are becoming disturbed. The stress put on natural resources negatively impacts the diversity of host plants preferred for infestation by parasitic plants (Skrypnik et al., 2020). Notwithstanding, we narrowed our study to host trees located in and nearer to one major city, and thus the findings could be biased by the monopolised human activities causing the disturbance in vegetation. To eliminate such biases, extensive research is recommended to compare the distribution of mistletoes on host plants found in disturbed and undisturbed sites far from the city. The limited sample size of the trees identified for mistletoe infestation in this study possesses a restricted representation of the mistletoe species richness. Additional surveillance of parasitic plants in the study areas would be preferable, as well over multiple seasons or years to determine general temporal and seasonal effects.

## Conclusion

The results from our study sites show a limited number of host tree species (10) from six families, with those belonging to Fabaceae constituting the most infested ones compared to other families. All the four mistletoe species identified, favoured generalist as opposed to specialist form of host tree infestation. The mistletoes found fall in two families, Loranthaceae and Viscaceae. The findings suggest P. kalachariensis (loranth) and V. rotundifolium (Viscaceae) as the most familiar parasites in urban and rural environments, respectively. In instances where multiple mistletoes infected a solitary host, most of the infestations involved a lorathoid species and Viscaceae member. The parasitic plant, V rotundifolium was seldom found in cohabitation; while P. kalachariensis was popular. The findings are supportive of our preliminary hypotheses: mistletoe infestations are

more prevalent in urban than rural sites, some host plants are highly preferred for infection over others, and in case of assemblage of a sole host by more than one parasite, the number of parasites would significantly differ. However, an additional in-depth study is required to define the level of success of these mistletoes on a loner host and to shed light on factors that code for the favour of one parasitic plant over the other under a cooccurrence state.

## **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The authors thank the Botswana University of Agriculture and Natural Resources for availing all equipment for use in this research project.

#### REFERENCES

Alias D, Milton S, Herrmann E, Seymour C (2003). A collation and overview of research information on *Boscia albitrunca* (shepherd's tree) and identification of relevant research gaps to inform protection of the species. Department of Water Affairs and Forestry, South Africa. Available at:

https://www.dffe.gov.za/sites/default/files/reports/bosciaalbitrunca researchinformation.pdf.

- Al-Robai SA (2023). Ecology and Diversity of Angiosperm Parasites and Their Host Plants along Elevation Gradient in Al-Baha Region, Saudi Arabia. Diversity 15(10):1065.
- Arsiso BK, Mengistu TG (2023). Land Use and Land Cover Change Modulates Hydrological Flows and Water Supply to Gaborone Dam Catchment, Botswana. Water 15(19):3364.
- Botswana S (2017). Botswana environment statistics. Water and Climate Digest. Available at https://www.statsbots.org.bw/sites/default/files/publications/Climate% 20Digest%20%202019.pdf.
- Bouwmeester H, Sinha N, Scholes J (2021). Parasitic plants: physiology, development, signaling, and ecosystem interactions. Plant Physiology 185(4):1267-1269.
- Casadesús A, Munné-Bosch S (2021). Holoparasitic plant-host interactions and their impact on Mediterranean ecosystems. Plant Physiology 185(4):1325-1338.
- Dean WRJ, Midgley JJ, Stock WD (1994). The distribution of mistletoes in South Africa: patterns of species richness and host choice. Journal of Biogeography 21(5):503-510.
- Dlama TT, Oluwagbemileke AS, Enehezeyi AR (2016). Mistletoe presence on five tree species of Samaru area, Nigeria. African Journal of Plant Science 10(1):16-22.
- Dzerefos CM, Witkowski ETF, Shackleton CM (2003). Host-preference and density of Woodrose-forming mistletoes (Loranthaceae) on savanna vegetation. South Africa. Plant Ecology 167(1):163-177.
- Erdogan P (2022). Parasitic plants in agriculture and management. Parasitic Plants 1. Available at: https://doi:10.5772/intechopen.98760
- Fontúrbel FE, Salazar DA, Medel R (2017). Why mistletoes are more aggregated in disturbed forests? The role of differential host mortality. Forest Ecology and Management 394:13-19.
- Gairola S, Bhatt A, Govender Y, Baijnath H, Procheş Ş, Ramdhani S (2013). Incidence and intensity of tree infestation by the mistletoe *Erianthemum dregei* (Eckl. and Zeyh.) V. Tieghem in Durban, South Africa. Urban Forestry and Urban Greening 12(3):315-322.

Grímsson F, Xafis A, Neumann FH, Scott L, Bamford MK, Zetter R

(2018). The first Loranthaceae fossils of Africa. Grana 57(4):249-259. Godschalk SKB (1983). The morphology of some South African mistletoe fruits. South African Journal of Botany 2(1): 52-56.

- Griebel A, Watson D, Pendall E (2017). Mistletoe, friend, and foe: synthesizing ecosystem implications of mistletoe infection. Environmental Research Letters 12(11): 115012.
- Hishe M, Abraha B (2013). Mistletoe infection of woody plant species at Bahir Dar University main campus, Bahir Dar, Ethiopia. SINET: Ethiopian Journal of Science 36(2):119-124.
- Karlin MS, Karlin UO (2018). Ecology of Acacieae. Acacia: Characteristics, Distribution and Uses. pp. 27-81. Available at: https://www.researchgate.net/publication/327634592
- Krasylenko Y, Kinge TR, Sosnovsky Y, Atamas N, Tofel KH, Horielov O, Rambold G (2022). Consuming and consumed: Biotic interactions of African mistletoes across different trophic levels. Biotropica 54(4):1103-1119.
- Ma R, Miao N, Zhang H, Tao W, Mao K, Moermond TC (2020). Generalist mistletoes and their hosts and potential hosts in an urban area in southwest China. Urban Forestry and Urban Greening 53:126717.
- Madisa ME, Assefa Y, Kelemoge OD, Mathowa T, Segwagwe AT (2017). Incidence and level of mistletoe infestation in tree species at Botswana University of Agriculture and Natural Resources, Sebele Content Farm Campus, Botswana. International Journal of Environmental and Agriculture Research 3:53-58.
- Magrach A, Rodríguez-Pérez J, Piazzon M, Santamaría L (2015). Divergent effects of forest edges on host distribution and seed disperser activity influence mistletoe distribution and recruitment. Journal of Ecology 103(6):1475-1486.
- Makhabu SW, Tshireletso K, Setlalekgomo MR, Kgosiesele E, Akanyang L, Legwatagwata B, Nsinamwa M, Seletlo Z, Majaga S (2021). Herbaceous communities' heterogeneity in the Kalahari sandy soils environment: A case study of Kweneng, Kgatleng Districts and Mahalapye Sub-District of Botswana. Journal of Biodiversity and Environmental Sciences18(6):87-101.
- Matthies D (2021). Closely related parasitic plants have similar host requirements and related effects on hosts. Ecology and Evolution 11(17):12011-12024.
- Mokgolodi NC, Hu Y, Shi LL, Liu YJ (2011). *Ziziphus mucronata*: an underutilized traditional medicinal plant in Africa. Forestry studies in China 13:163-172.
- Mosha AC (2016). Urban agriculture in Botswana. Commonwealth Journal of Local Governance (18):48-67.
- Motlhanka DMT, Motlhanka P, Selebatso T (2008). Edible indigenous wild fruit plants of eastern Botswana. International Journal of Poultry Science 7(5):457-460.
- Muche M, Muasya AM, Tsegay BA (2022). Biology and resource acquisition of mistletoes, and the defense responses of host plants. Ecological Processes 11(1):24.
- Musselman LJ, Rodenburg J (2023). Parasitic Plants in African Agriculture. CABI. Available at: https://www.echocommunity.org/en/resources/97670675-53b4-40ca-833a-3c35bc92b0dc
- Niu Y, Laffitte B, Zuoqiu S, Seyler BC, Ha Z, Chen J, Tang Y (2024). Understanding predictors of mistletoe infection across an urban University campus in Southwest China. Urban Ecosystems pp. 1-15. Available at: https://doi.org/10.1007/s11252-024-01514-4
- Norton DA, Carpenter MĀ (1998). Mistletoes as parasites: host specificity and speciation. Trends in Ecology and Evolution 13(3):101-105.
- Okubamichael DY, Griffiths ME, Ward D (2011). Host specificity, nutrient and water dynamics of the mistletoe *Viscum rotundifolium* and its potential host species in the Kalahari of South Africa. Journal of Arid Environments 75(10):898-902.

- Okubamichael DY, Griffiths ME, Ward D (2016). Host specificity in parasitic plants-perspectives from mistletoes. AoB Plants 8. Available at: https://doi.org/10.1093/aobpla/plw069
- R Core Team (2013). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available at: http://www.R-project.org/
- Roxburg L, Nicolson SW (2005). Patterns of host use in two African mistletoes: the importance of mistletoe-host compatibility and avian disperser behaviour. Functional Ecology. pp. 865-873. Available at: https://doi: 10.1111/j.1365-2435.2005.01036.x
- Setshogo MP (1998). Setswana Plant Names and the Identification of Local Flora. Botswana Notes and Records 30(1):57-66.
- Setshogo M (2005). Preliminary checklist of the plants of Botswana. Southern African Botanical Diversity Network Report No. 37. Available at: https://www.sanbi.org/documents/sabonet-report-no-37preliminary-checklist-of-the-plants-of-botswana.
- Skrypnik L, Maslennikov P, Feduraev P, Pungin A, Belov N (2020). Ecological and Landscape Factors Affecting the Spread of European Mistletoe (*Viscum album* L.) in Urban Areas (A Case Study of the Kaliningrad City, Russia). Plants 9(3):394.
- Teixeira-Costa L, Davis CC (2021). Life history, diversity, and distribution in parasitic flowering plants. Plant Physiology 187(1):32-51.
- Těšitel J (2016). Functional biology of parasitic plants: a review. Plant Ecology and Evolution 149(1):5-20.
- Těšitel J, Li AR, Knotková K, McLellan R, Bandaranayake PC, Watson DM (2021). The bright side of parasitic plants: what are they good for? Plant Physiology 185(4):1309-1324.
- Tizhe TD, Yusuf CS, Timon D, Zakawa NN, Gaman M (2020). Mistletoe Species Richness and Rate of Infestation on the Tree Species in the Campus of Adamawa State University, Mubi, Nigeria. Asian Journal of Research in Botany 4(2):33-38.
- Tshireletso K, Makhabu SW, Nsinamwa M, Kgosiesele E, Setlalekgomo MR, Seletlo Z, Majaga S, Legwatagwata B (2018). Diversity patterns of woody vegetation of Kgatleng District, Botswana. Available at: https://doi: 10.7809/b-e.00353
- Twyford AD (2018). Parasitic plants. Current Biology 28(16):R857-R859.
- Valle ACV, de Carvalho AC, Andrade RV (2021). Viscum albumliterature review. International Journal of Scientific Research 10:63-71. Available at: https://doi: 10.21275/SR21430224614
- Van WB (2013). Field guide to trees of southern Africa. Penguin Random House, South Africa.
- Veste M (2007). Parasitic flowering plants on Euphorbia in South Africa and Namibia. Euphorbia World 3(3):1-9.
- Wahyuningtias S, Mardiastuti A, Mulyani YA (2021). Diversity of mistletoes and their distribution in Dramaga Campus, West Java, Indonesia. IOP Conference Series: Earth and Environmental Science 948. Available at: https:// doi:10.1088/1755-1315/948/1/012015.
- Walker JG, Hurford A, Cable J, Ellison AR, Price SJ, Cressler CE (2017). Host allometry influences the evolution of parasite hostgeneralism: theory and meta-analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 372(1719).
- Watson DM, Cook M, Fadini RF (2020). Towards best-practice management of mistletoes in horticulture. Botany 98(9):489-498.
- Yoshida S, Cui S, Ichihashi Y, Shirasu K (2016). The Haustorium, a Specialized Invasive Organ in Parasitic Plants. Annual Review of Plant Biology 67:643-667.