Indirect interactions between elephants (*Loxodonta africana*) and mopane caterpillars (*Imbrasia belina*) through their shared food resource – mopane trees (*Colophospermum mopane*).

by

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Summary

Mopane (*Colophospermum mopane*) trees are browsed upon by two key species, namely mopane caterpillars (*Imbrasia belina*) and African elephants (*Loxodonta africana*), which each inflict a different type of damage while feeding, namely defoliation (leaf removal) and pruning (branch and/or stem breakage). Damage type can have a significant influence on plant responses, and these induced changes in morphological and chemical characteristics of regrowth can influence the subsequent feeding behaviour by each species. The objective of this study was therefore partly to investigate the differential effect of defoliation by mopane caterpillars and pruning by elephants on mopane trees, and then to investigate whether these two taxonomically different species interact through their shared food resource, by looking specifically at the effect of elephant utilisation of mopane on mopane caterpillar abundance.

To determine the comparative effect of each browsing type, mopane trees were subjected to simulated mopane caterpillar or elephant utilisation treatments, at various frequencies and times within the year. Regrowth characteristics were then measured on treatment and control trees, as well as on naturally utilised and unutilised trees. Reproductive investment was also recorded on naturally utilised and unutilised trees. Additionally, the impact of mopane caterpillar defoliation and elephant pruning on plant stress was investigated by measuring the level of fluctuating asymmetry (FA) in leaves. Then, to determine whether there is an interaction between elephants and mopane caterpillars, mopane caterpillar egg mass abundance in areas of high elephant impact was compared to that in areas of low elephant impact. Firstly, however, in areas without elephant damage, those tree characteristics determining host tree preference by ovipositing mopane moths were identified. From this, an understanding of how elephant utilisation may influence mopane caterpillar abundance could therefore be gained.

Defoliation and pruning had a significant different effect on mopane regrowth responses. Shoot and leaf length were significantly longer on pruned trees than control trees, for both naturally utilised and simulated elephant treatment trees, while there was no difference in shoot density. Defoliation, however, resulted in shorter shoots and leaves, particularly on naturally defoliated trees, which also had leaves of a higher nutritional value (tannin:protein ratio and total polyphenolic content) than control trees. A similar increase in leaf nutritional value was recorded in areas of high elephant impact in the Kruger National Park, but not after simulated or natural elephant damage in Venetia, where natural elephant utilization was less intense. Time since damage (i.e. first versus second flush) had a significant influence on regrowth after pruning, as shoot and leaf length were significantly longer on trees flushing for the first time, while within-season timing of damage was important for defoliation, as late-season defoliation had a greater negative impact than mid-season defoliation. Late-season defoliation also had a negative effect on leaf carriage into the dry season, while pruning appeared to aid leaf retention.

Reproductive investment was found to be unaffected by mopane caterpillar defoliation or elephant pruning, as mean pod density and pod mass on utilised trees was no different to unutilised trees. Defoliation also had no influence on a plant's likelihood of flowering that same season, with flowering being determined more by tree height. Unlike pod production, however, mean leaf density was significantly reduced in the regrowth of defoliated trees, presumably due to the use of stored resources for reproduction prior to the onset of regrowth.

Neither simulated nor natural defoliation by mopane caterpillars and pruning by elephants was found to affect the level of leaf FA in mopane trees, even though the degree of damage inflicted on trees was considerably higher than in studies on other species where increases in FA were observed. Mopane therefore appears to be extremely tolerant of herbivory in comparison to other species. A positive relationship between leaf nutritional value (higher protein and lower tannin and polyphenolic content) and FA was detected, but only when trees from all study areas (i.e. a wide range of environmental conditions) were considered simultaneously. Environmental conditions, rather than herbivory, therefore appear to have a greater stressing affect on mopane.

In the absence of heavy elephant utilisation of mopane trees, tree size, rather than shoot length, leaf length, leaf FA or leaf nutritional value, was found to have the greatest influence on oviposition behaviour of mopane moths. Ovipositing moths showed a preference for the tall riverine habitat over the shorter woodland and scrub mopane. This preference for large trees was, however, not evident at the individual tree level, as even though egg mass number per tree was positively related to tree height, large trees were not utilised more than expected according to the available canopy volume in each size class (resource availability).

Heavy elephant utilisation of mopane had a negative impact on the density of tall trees within an area, due to branch and stem breakage while feeding. Unsurprisingly then, mopane caterpillar egg mass abundance was also significantly reduced in these areas, even though the nutritional value of leaves was higher than in non-elephant impacted areas. Elephants therefore appear to have a negative effect on mopane caterpillar abundance, primarily due to their negative impact on the density of tall mopane trees. This megaherbivore and invertebrate do therefore interact through their shared food resource, mopane trees.

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CHAPTER 1

INTRODUCTION

Colophospermum mopane (commonly known as 'mopane') is a xeric savanna woodland species that dominates over vast areas of land in southern Africa, where it out-competes most other woody species within its range and generally forms monospecific stands (Timberlake 1995; O'Connor 1999). Its foliage is an important browse for many mammalian herbivores such as eland (*Taurotagus oryx*), kudu (*Tragelaphus strepsiceros*), and impala (*Aepyceros melampus*; Styles 1993; Styles & Skinner 2000), as well as a number of invertebrates such as puss moth larva (Family: Notodontidae, Order: *Epicerura*) and the mopane psyllid (*Retroacizzia mopanei*; Picker *et al*. 2002). The two main herbivore species associated with mopane, however, include a megaherbivore, the African elephant (*Loxodonta africana*), and an invertebrate, the mopane caterpillar (*Imbrasia belina*).

Elephants utilize mopane predominantly through branch stripping and stem breakage (thereby having a 'pruning' affect) and show a preference for the species, despite it's availability (Smallie & O'Connor 2000). Consequently, their browsing can have a significant impact on the vegetation structure (similar to that caused by fire, Kennedy & Potgieter 2003), thereby making them a keystone species in these woodlands (Timberlake 1995). Although not agents of such structural change, mopane caterpillars are also well known for their utilization of mopane trees, as large population outbreaks are common within mopane woodland, often resulting in vast stands of trees being completely defoliated (Ditlhogo 1996). These two browsers therefore not only share the same food resource, but also both have a significant impact on the vegetation. Hence, it is expected that feeding by the one species could have a significant influence on feeding by the other.

Considering the interaction between these two browsers is unique to most other intra-guild studies, as here the two key species sharing the same resource are taxonomically so different. Other examples of inter-taxon studies include the interaction between birds and mammals (Brown *et al*. 1997), and ants and rodents (Davidson *et al*. 1984), yet both these studies looked at seed utilization, not browsing. The interaction between browsers feeding on different plant parts has also been documented, such as pocket gophers (*Thomomys talpoides*) feeding on below-ground root material and insects feeding on above-ground foliage (Ostrow *et al*. 2002). Furthermore, interactions between browsers utilising the same resource have been documented, yet these studies have tended to focus on taxonomically similar species (Bryant 2003). This study, however, adopts a novel approach and considers interactions within a functional group (the above-ground browsing guild), instead of the more traditional similar-species approach.

Defoliation by mopane caterpillars takes place only once or twice a year during November/December and possibly again in February/March, depending on the rainfall. Branch breakage by elephants, however, occurs mainly at the end of the dry season (August) when other resources are most limited (Lewis 1986). A direct interaction between the two browsers is hence not likely, as their timing of mopane utilization is different. Instead, an indirect interaction could occur, through the impact on plant responses by each species.

Woody species respond differently to herbivory according to the frequency, intensity, timing and type of damage (Maschinski & Whitham 1989; Riba 1998; Tiffin 2002). The most dramatic difference between elephant and mopane caterpillar browsing, however, is the type of damage inflicted, namely: pruning versus defoliation. Studies have shown that trees respond differently to each type of damage, due to the difference in the type and quantity of plant tissue removed. Pruning, which removes branch ends, tends to result in an increased production of side shoots, as lateral meristems are no longer kept dormant by the dominant apical mersitem (Honkanen & Haukioja 1994). Additionally, an increase in shoot length and leaf size tends to occur, as according to the resource availability hypothesis, the reduction in tree size caused by branch removal results in a greater availability of stored resources for regrowth on remaining branches (Danell *et al*. 1994; Duncan *et al*. 1998; Bergström *et al*. 2000; Lehtilä *et al*. 2000). Defoliation on the other hand, which has no effect on plant size but potentially a negative effect on stored resources (if resources used for the initial flush are not yet replenished), characteristically results in regrowth with smaller and/or fewer shoots and leaves (Gadd *et al*. 2001; Anttonen *et al*. 2002; Piene *et al*. 2002). In addition, the chemical composition of foliage can be differentially affected by defoliation and pruning. Often associated with the increased growth of leaves after pruning, is a decrease in the production of defensive secondary compounds, as these are expensive to produce and slow down growth (Herms & Mattson 1992). Defoliation, however, has a variable effect on foliage quality, which tends to be related to plant growth rate (Bryant *et al*. 1991).

Despite the numerous studies on plant responses to herbivory, the true comparative effect of defoliation versus pruning is not yet fully understood, as responses to herbivory also vary between species according to their growth strategy (deciduous versus evergreen; Krause & Raffa 1996) and few studies have made comparisons within an individual tree species. By determining the differential impact of pruning and defoliation on mopane regrowth alone, we could therefore significantly improve our understanding of how an individual species has adapted to persist while exposed to two such different damage types. For example, does the species display tolerance and/or resistance to herbivory (Rosenthal & Kotanen 1994; Mauricio *et al*. 1997; Purrington 2000; Stowe *et al*. 2000)?

In addition, knowledge of regrowth responses is necessary to be able to understand the interaction between mopane caterpillars and elephants, as induced morphological and chemical characteristics of regrowth can influence the subsequent feeding behaviour by browsers (Bryant *et al*. 1991; Coley & Barone 1996; Cooper *et al*. 2003). The preference by moose (*Alces alces*) for previously browsed birch trees (*Betula pendula* and *B. pubescens*) is, for example, thought to be due to the greater long-shoot size on these trees, which facilitates a higher cropping rate (Danell *et al*. 1985). Similarly, elephants tend to prefer mopane trees previously utilised by them, as the damage-induced coppicing shoots provide a greater availability of their preferred shoot size (Smallie & O'Connor 2000). Insects also tend to select host plants according to their regrowth characteristics, and in the few studies looking at mammal-insect browser interactions, insect abundance was indeed influenced by previous mammalian browsing. Here, changes in leaf nutritional value is often an important host choice determinant, as this can potentially influence offspring performance, either through an increased growth rate or a decrease in predation by the sequestering of biologically active chemicals into their own tissue or glands (Karban & Agrawal 2002). For example, the abundance of leaf-eating insects was greater on birch (*Betula pubenscens*) trees previously browsed by moose to a moderate degree, which had a higher nitrogen content than leaves on lightly browsed trees (Danell & Huss-Danell 1985); while leaf beetles (*Chrysomela confluens*) were more abundant on the regrowth of cottonwoods (*Populus fremontii* and *P. angustifolia*) previously cut by beavers (*Castor Canadensis*), that had increased levels of defensive chemicals (which they sequestered for defensive purposes; Martinsen *et al*. 1998).

Any elephant induced changes to mopane trees, particularly the leaves, is therefore expected to have an influence on ovipositing behaviour by mopane moths. Similarly, the predicted decrease in shoot and leaf size after mopane caterpillar defoliation and a possible decrease in leaf quality may have a negative effect on elephant browsing, yet an increase in leaf quality could have a positive effect. Even though it is clearly possible for a two-way interaction to exist between these two browsers, it is however unfortunately beyond the scope of this project to investigate the interaction in both directions. Consequently, only the effect of elephant browsing on mopane caterpillar abundance is addressed here.

In addition to plant regrowth responses, another factor affected by herbivory is reproduction. Similar to regrowth, factors influencing reproductive responses include: the intensity, timing, type and frequency of herbivory (Maschinski & Whitham 1989; Doak 1992, Marquis 1992); resource availability (Lennartsson *et al*. 1998); inter and intra-species competition (Tiffin 2002); and the characteristics of the plant species. Timing may be important, as the period of time between when damage occurs and the onset of flowering could affect the amount of stored resources utilised for regrowth rather than reproduction. When the forest shrub *Piper arieianum* was subjected to simulated defoliation three months before flowering, for instance, seed production decreased significantly, but when defoliated at the time of flowering, no difference in seed production was recorded (Marquis 1992). Similarly, the type of damage also influences the allocation of resources towards reproduction within plants. For example, Strauss (1991) found that the number of smooth sumac (*Rhus glabra*) stems producing fruits increased after pruning by whitetail deer (*Odocoileus virginianus*), but decreased after leaf damage by a specialist chrysomelid beetle (*Blepharida rhois*). The response to each damage type is variable, however, as decreased reproduction after pruning (Peinetti *et al.*

2001) and no change in reproduction after defoliation has also been reported (Meyer 2000). Clearly, plant tolerance of herbivory is therefore not only determined by regrowth responses, but also by the ability to maintain a certain reproductive rate and invest in future generations.

Flowering in mopane takes place predominantly in January and February (Smit 1994), which is just after the first defoliation event by mopane caterpillars in November/December, but about four months after the main period of pruning by elephants in August. Timing, together with damage type may therefore be expected to influence the degree of impact elephant and caterpillar utilization have on reproduction in mopane trees. Determining this differential impact would then help our understanding of the impact each browser has on mopane tree fitness. Ultimately, this could also reveal the long-term interaction between the two browsers, as changes in reproduction could affect plant recruitment and consequently, tree density and resource availability.

While measures of regrowth and reproduction indicate how a plant has adapted to withstand different types of herbivore damage, the difference in response to pruning and defoliation makes it difficult to determine the actual comparative stress on the plant. Compensatory responses could, for example, mask the more long term detrimental effects of browsing, as damaged plants could initially perform as well as or better than non-damaged controls. Instead, developmental instability (DI) is therefore often used as an indicator of stress. Fluctuating asymmetry (FA), which reflects small random deviations from symmetry in otherwise bilaterally symmetrical characters (Palmer & Strobeck 1986), is the most commonly used measure of DI. Since the development of the right and left sides of a bilaterally symmetrical morphological character are controlled by the same genes, the degree of FA is thought to represent the inability of an individual to control developmental processes under given environmental conditions (Møller 1995; Møller &

de Lope 1998). FA is therefore considered a useful and objective tool for measuring stress levels in both plants and animals, where in most cases, an increase in asymmetry is directly related to a decrease in growth, fecundity and survival (Møller 1997; Møller 1999).

Plants are considered highly suitable organisms for studying developmental instability, due to their modular structure that results in repeated structures that reflect developmental performance (Freeman *et al*. 1993). In perennial woody plants, the character most commonly used to measure stress is foliar FA (e.g. Zvereva *et al*. 1997; Martel *et al*. 1999), for which mopane is particularly suitable, as leaves are pinnate, with two large leaflets. The level of leaf FA in mopane trees previously utilised by elephants and mopane caterpillars could therefore be a useful indicator of which browsing type causes the most stress on the trees. Furthermore, developmental instability is sometimes associated with increased leaf nutritional value (due to accelerated growth in response to browsing, Martel *et al*. 1999), and plants with a higher degree of FA may therefore be more susceptible to further herbivory (Møller 1995). Leaf FA may then also be used as an indicator of mopane leaf chemistry, which in turn could indicate whether host tree preference by ovipositing mopane moths is related to leaf chemistry (i.e. plant stress).

The objective of this study was therefore partly to investigate the differential effect of defoliation by mopane caterpillars and pruning by elephants on mopane trees, determined by measures of: (1) regrowth responses, (2) reproduction and (3) leaf fluctuating asymmetry. Additionally, the interaction between elephants and mopane caterpillars through this shared food resource was investigated, by looking specifically at whether browsing by elephants has an effect on mopane caterpillar abundance.

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CHAPTER 2

STUDY SITES AND SPECIES

2.1 Study sites

2.1.1 Venetia-Limpopo Nature Reserve

The Venetia-Limpopo Nature Reserve (Venetia) is situated in the Northern Province of South Africa (22°08_ – 27_S and 29°13_ – 28_E), slightly south of the meeting point of South Africa, Zimbabwe and Botswana (Fig. 2.1). It is a privately fenced park and is 34 500 ha in extent.

This semi-arid savanna is characterised by wet, hot summers (monthly mean maximum 32°C from October to December) and dry, mild winters (monthly mean maximum 24.7°C in June). The mean annual rainfall for Pontdrift, about 15 km from the reserve, is 366 mm (1967-1997) with a 36% coefficient of variation. The rainy season usually extends between October and March, with the probability of rain during January being higher than for other months (Smit & Rethman 1998a; Fig. 2.2 & 2.3). Rainfall occurs mainly in the form of thunder-showers.

The topography of the area is predominantly flat, with sandstone as the dominant underlying bedrock beneath deep (> 2 m) colluvial soils. The occasional rocky sandstone outcrop interrupts the landscape, together with two major seasonal rivers: the Kolope (flowing south to north) and the Setonki (west to north). Alluvial soils are found adjacent to rivers.

From the 1940s the land was used for livestock ranching. Farms were heavily stocked with cattle and goats, resulting in soil and vegetation degradation (MacGregor & O'Connor 2002). Livestock were removed when De Beers Consolidation Mines Ltd. purchased the land between 1981 and 1996 and the area was consolidated as a nature

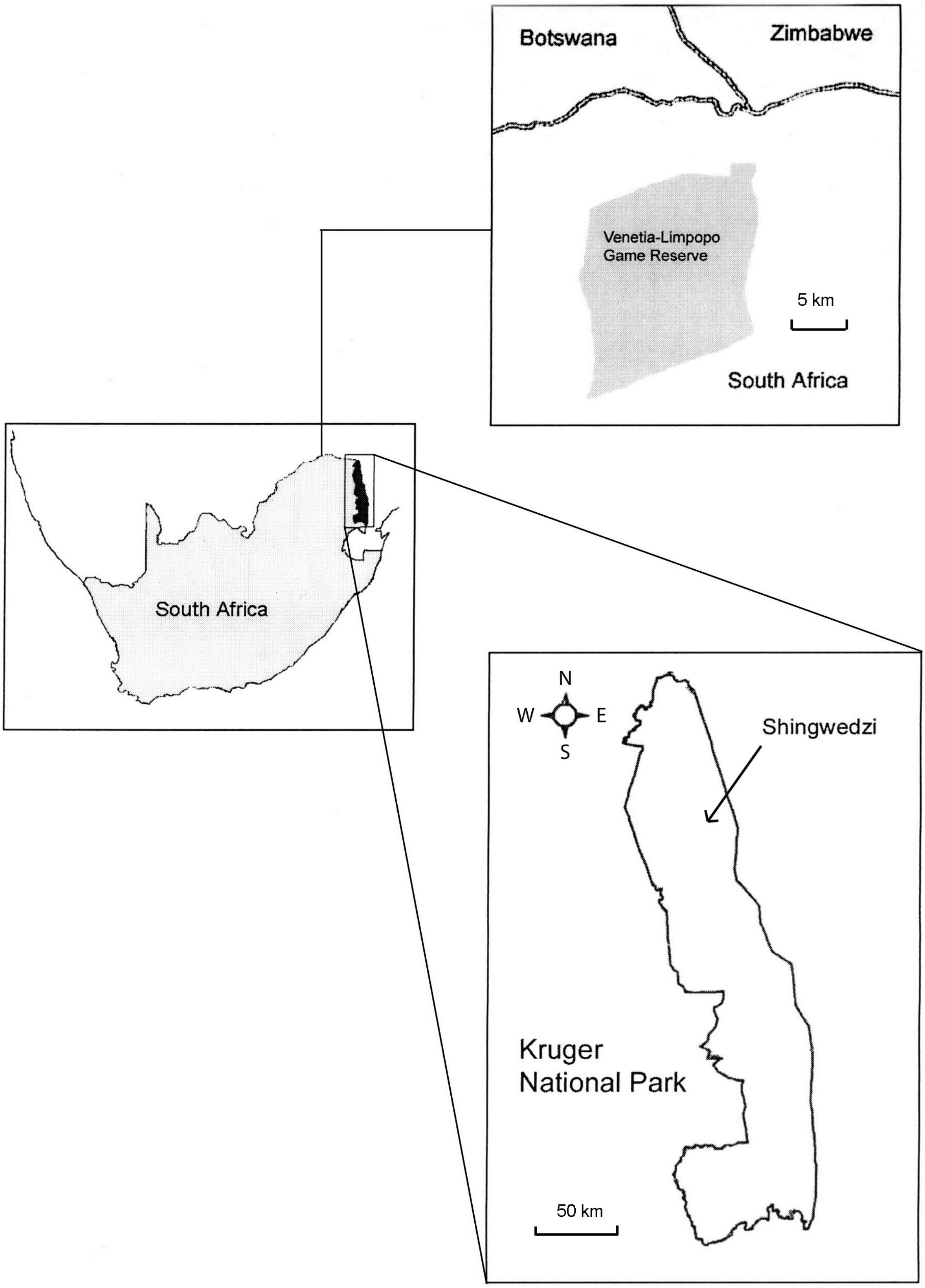

Figure 2.1 Maps showing the location of the two study sites used in this study, namely the Venetia-Limpopo Nature Reserve and Kruger National Park.

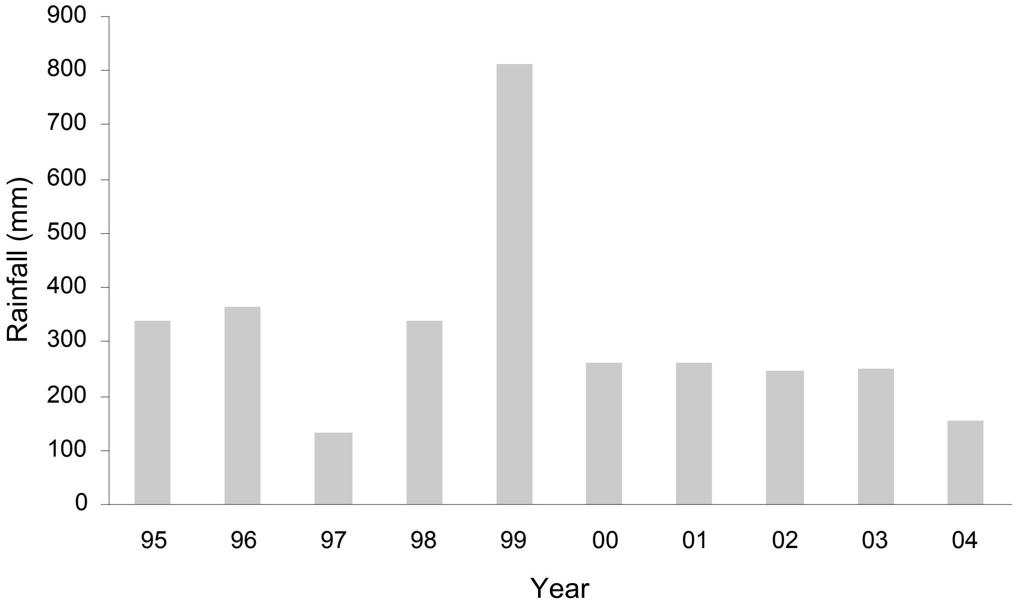

Figure 2.2 Annual seasonal rainfall (July- June) for Venetia from July 1995 to June 2005 (year indicates start of season).

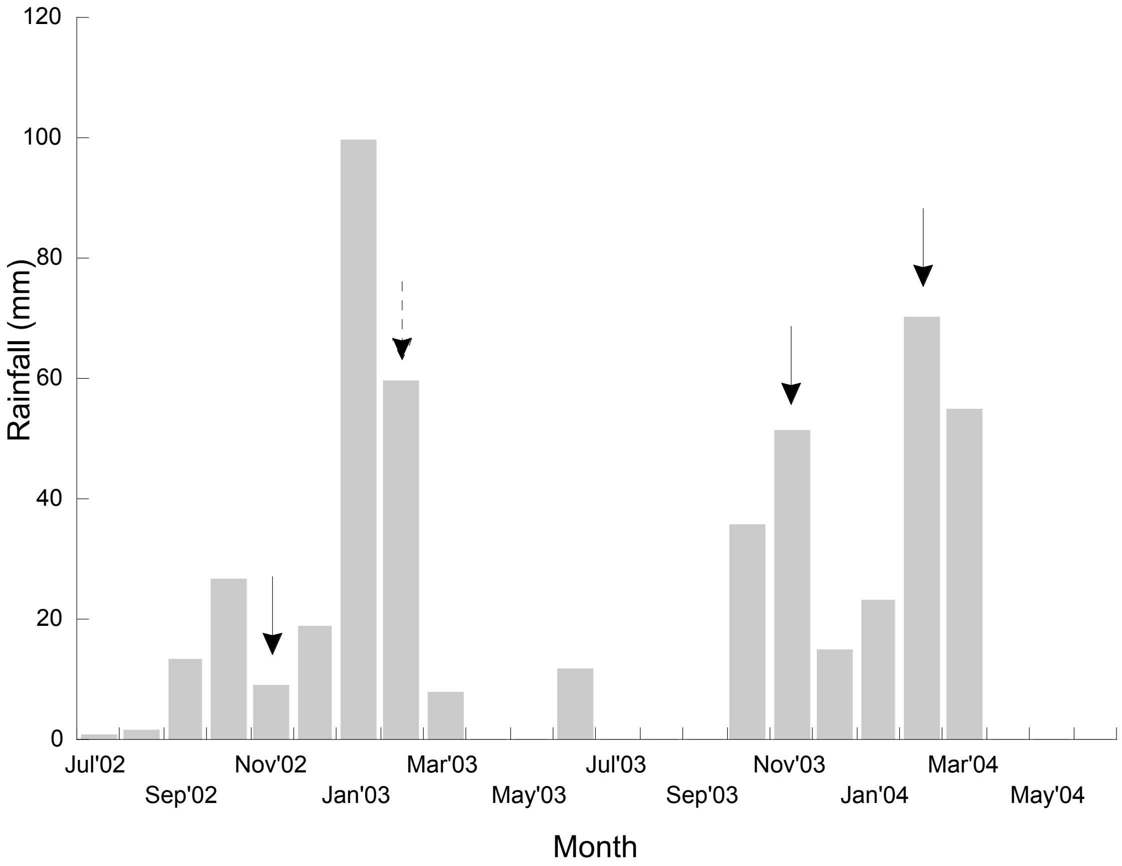

Figure 2.3 Monthly rainfall in Venetia over the two year period during which the study took place. Arrows indicate when oviposition by mopane moths took place (dashed arrow represents very few moths).

reserve. Indigenous herbivores were reintroduced in 1989 -1991, including 34 elephants in 1993 and 1994. The elephant population, which is now estimated to be at 60, is still at a relatively low density (0.17 animals/km^2) compared to other populations within mopane woodland (e.g. 1.1 elephants/km^2 in Luangwa Valley, Zambia; Lewis 1991).

Vegetation of the region is dominated by *Colophospermum mopane* (commonly known as 'mopane') and is classified as 'Mopane Veld' (Acocks 1953). Sixteen of the eighteen vegetation types in Venetia are dominated by this species, with short stature mopane woodland (average height of 1.5 m), found on colluvial soil, dominating (O'Connor 1992). These woodlands are dense, with > 70% cover. Within riverine vegetation, mopane trees reach up to 10 m in height. Other common tree species within Venetia include *Boscia foetida*, *Salvadora angustifolia* and *Lycium austrinum*.

2.1.2 Kruger National Park

The Kruger National Park (Kruger) is situated in the lowveld of northeastern South Africa, bordering Mozambique in the east and touching Zimbabwe in the North (Fig. 2.1). It is an elongated park extending about 350 km from north to south with an average width of 60 km, covering about two million hectares.

An aspect of this study was carried out in the north of Kruger, around Shingwedzi (23.10°S and 31.43°E). The topography of the area is flat, with basalt rock as the underlying bedrock in the east and granite in the west. Bands of alluvial soils following drainage lines run through the area. Shingwedzi falls within the northern arid bushveld zone and is also characterized by wet, hot summers (mean monthly maximum 34ºC in January) and dry, mild winters (mean monthly maximum 26ºC in June). The mean annual rainfall is 400 mm and is strongly seasonal, falling predominantly between October and May (Venter *et al*. 2003; Fig. 2.4). A number of seasonal rivers run through the

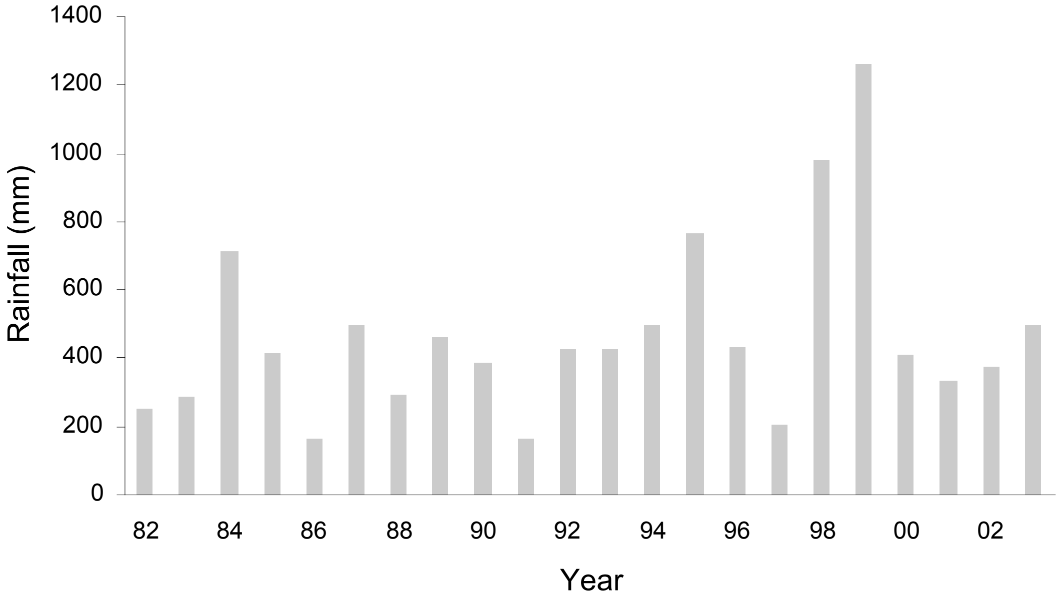

Figure 2.4 Annual seasonal rainfall (July- June) for Shingwedzi in the Kruger National Park from July 1982 to June 2003 (year indicates start of season).

Shingwedzi area, and permanent surface water is available through numerous artificial water points (boreholes and dams) constructed in 1933 and in the 1950s and 1960s (Gaylard *et al*. 2003). As in Venetia, the vegetation is dominated by mopane trees, which range in height from short scrub mopane to taller riverine trees. The alluvial plains are more species-rich, however, with tree species such as jackal-berry (*Diospyros mespiliformis*), leadwood (*Combretum imberbe*) and the common cluster fig (*Ficus sycamorus*).

Between 1836 and 1902, uncontrolled hunting and the rinderpest epizootic outbreak in 1896 decimated game populations within the Kruger area. By 1903 most of the area became a game sanctuary where until 1926, when it was officially proclaimed the Kruger National Park, the emphasis was on the protection and rebuilding of game populations (Mabunda *et al*. 2003).

Historically, elephant numbers are believed to have been low in the area (Whyte 2001), but by 1896 they were extinct. The first subsequent elephant sighting in Kruger was in 1905 in the southern region, from where they spread north and were first sighted around Shingwedzi in 1933. Their numbers increased rapidly until 1967, when 6, 586 individuals were counted, after which culling began. Between 1967 and 1994 (when culling was suspended), a total of 16, 201 elephant had been killed or removed from the park, and between 1994-2001 a further 465 individuals were removed. By 2002 the population had reached about 10, 459 (Whyte *et al*. 2003).

2.2 Mopane trees

2.2.1 Distribution and ecology

Colophospermum mopane (Caesalpinioideae, Leguminosae), a xeric savanna woodland species, is the dominant tree over vast areas of land in southern Africa with an altitude range of 300-1,000 m. Occurring over approximately 550, 000 km^2, it's range includes Mozambique, Zimbabwe, Angola, Botswana, Namibia, South Africa and southern Malawi (Fig. 2.5; Mapaure 1994). Factors determining the distribution of mopane are thought to vary in different parts of its range, but generally include frost, soil type, minimum rainfall and length of the growing season (Timberlake 1995).

Physiologically, the species is well adapted to dry conditions, with the annual (unimodal) rainfall over mopane veld averaging 300-700 mm, with a long dry season. There is also an association with comparatively clay-rich soils with a high pH (Timberlake 1995). According to Gertenbach (1987) mopane trees do not favour alkaline soils *per se*, as they will grow better on fertile, slightly acid permeable soil. They are, however, most often excluded from these soils by deep-rooted *Acacia* species (Cole 1986). Mopane tend to thrive on soil where the exchangeable calcium in the B-horizon is high, and where the B-horizon is shallow (Gertenbach 1987). The ability to grow so well under such soil conditions, and resist drought (O'Connor 1999), is mainly due to the root system that is markedly shallow and extensive (usually 300-1200 mm deep), extending deeper in deep soils (Smit 1994; Timberlake 1995). Additionally, the root biomass is exceptionally high (mean: 17 354 kg ha^{-1}), exceeding the leaf biomass (mean: 1 023 kg ha^{-1}; Smit & Rethman 1998b), and the roots of mopane are also able to utilize soil water at a low metric potential (below –15.2 bar, Henning & White 1974). This gives them a competitive advantage over grasses and may explain the poor grass layer in most mopane woodland (O'Connor 1999). The large underground biomass also enables mopane to

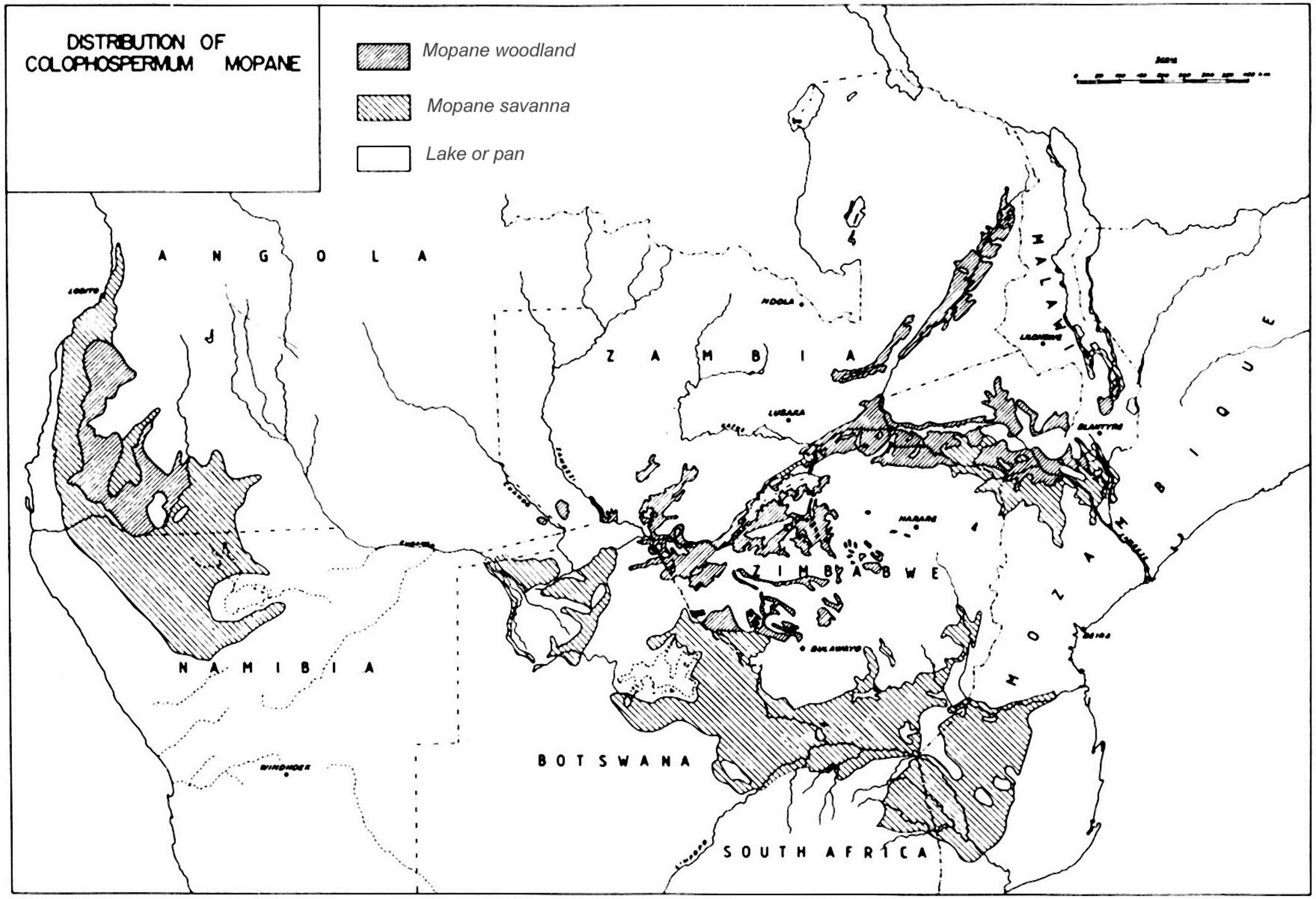

Figure 2.5 Distribution of *Colophospermum mopane* in Southern Africa (from Mapaure 1994).

readily produce shoots from the rootstock when the plant is damaged by fire, drought or herbivory.

It is common for mopane to out-compete most other woody species within its range (O'Connor 1999) and generally occurs in monospecific stands with comparatively low alpha and gamma diversities (Timberlake 1995). There is, however, a high degree of variability within mopane woodlands. While mopane usually ranges from about 10 m in height, with large areas of low scrub of 1-2 m, stands of trees up to 20 m high exists and are termed "cathedral mopane" (Van Wyk 1993). O'Connor (1992) identified the principle cause of these variations in tree height to be variations in the soil, particularly depth and pH. In the Kruger National Park, mopane growing on soils derived from basic material i.e. basalt, dolerite and gabbro are multi-stemmed shrubs of 1-2 m in height, while trees growing on sandy soils are usually single-stemmed and up to 5 m tall (Fraser *et al.* 1987). Mopane woodlands of stunted growth are generally found on soil with a high clay content, relatively impenetrable B-horizon, sodic soil conditions and a shallow A-horizon (Dye & Walker 1980). Densities of mature mopane woodland also vary, ranging from a few trees per hectare in arid northwestern Namibia (Viljoen 1989) to 481 trees ha^{-1} in southeastern Zimbabwe (Kelly & Walker 1976) and 2,740 trees ha^{-1} in northern South Africa (this study). An additional factor known to influence tree density and structure in mopane woodlands is fire. Tree density and tree height can be significantly reduced in frequently burnt areas, while the number of stems per tree tends to increase due to coppicing (Kennedy & Potgieter 2003).

2.2.2 Phenology

Mopane is a slow-growing deciduous species with pinnate leaves, consisting of two large leaflets that together resemble the wings of a butterfly. Leaf size may vary considerably on the same tree (Wiggins 1997; Potgieter & Wessels 1998) and under hot, dry conditions, the leaflets fold closed and hang down, thereby casting very little shade. Trees are generally leafless from August to October, after dropping their leaves from the onset of the dry season. This is, however, dependant on rainfall (amount and distribution), as leaves may be retained between successive rainy seasons under favourable conditions, which can then be an important source of browse at a time when resources are most limited (Oates 1972). Leaf flush may occur after the first summer rains in October and November (in South Africa) or independently of rain (Styles & Skinner 1997a).

Flowers are small, greenish in colour and inconspicuous (Krüger *et al*. 1999), emerging after the leaf flush, from December to March. Their emergence is, however, highly irregular with some trees not flowering for several years at a time. Fruits of mopane are indehiscent, flat and kidney-shaped with a single yellowish and wrinkled seed within the pod (Coates Palgrave 1983). The seed is covered with resinous glands, making it sticky, yet fruits are dispersed by rainwater and wind (not by epizoochory; Styles & Skinner 1997b).

2.2.3 Uses

Mopane woodland is of great economic importance in the areas where it occurs. Large parts of mopane woodland are used for game and livestock farming (cattle and goats), by both commercial and rural farmers. Here, mopane forms a crucial role, as its foliage is an important browse for many herbivores such as elephant (*Loxodonta africana*), eland (*Taurotagus oryx*), kudu (*Tragelaphus strepsiceros*), and impala (*Aepyceros melampus*; Lewis 1986; Styles 1993; Ben-Shahar 1998; Styles & Skinner 2000). Leaves have a high crude protein content, varying from 9,3% in winter to 15.9% in summer. Considering the crude protein content of the diet of kudu, a highly selective feeder, is about 12-14% in the late wet season and 9% at the end of the dry season (Owen-Smith & Cooper 1989), the crude protein content of mopane clearly exceeds the requirements of even such a selective feeder. The total phenolic content of its leaves is also comparatively low, as Styles and Skinner (1997a) found young leaves to have a mean total phenolic content of 147.7 mg C./g dry material, which is significantly lower than that of *Acacia nilotica* (373,3 mg C./g), a palatable woody species (du Toit *et al.* 1990). Additionally, senescing leaves were found to be at their most palatable in late winter/early spring, thus providing a valuable resource for herbivores before the spring flush (Styles & Skinner 1997a). Seedpods are also eaten during the dry season, and in some areas, mopane has been known to prevent large-scale livestock and game losses from drought.

Within rural communities the tree is valued for its timber, which is used in the construction of huts, fencing and kraals (Madzibane & Potgieter 1999; Mashabane *et al.* 2001). Mopane is also extensively used as firewood, as the wood burns slowly and produces good coals (Tietema *et al.* 1991), and charcoal produced from mopane is widely utilized in countries like Zambia (Chidumayo 2000). Another valuable resource obtained from mopane woodlands is the mopane caterpillar (*Imbrasia belina*), which forms an important food source for rural communities. This edible caterpillar, which feeds almost exclusively on mopane and displays outbreak dynamics, is harvested in large numbers and has become an important trading commodity (Makhosandile 1999). The larvae are collected during the summer and are then gutted and dried for consumption. In Botswana, the mopane trade brings in about R46 million annually (Potgieter *et al.* 2001).

2.3 Mopane moths and caterpillars

Mopane caterpillars are of the emperor moth *Imbrasia belina*, a Saturnid. The species is widespread in southern Africa, tropical and East Africa, occurring from semi-desert to savanna and thick bush. The larvae feed on a number of tree species, including *Colophospermum mopane*, *Carrissa grandifola*, *Sclerocarya birrea*, *Commiphora glandulosa*, *Acacia tortilis*, *Cassia abreviata*, *Diospyros* spp., *Ficus* spp., *Rhus* spp., *Terminalia* spp. and *Trema bracteolate* (Pinhey 1972). However, large outbreak populations only follow that of their main host plant, mopane.

The life cycle of *I. belina* has been well documented (Ditlhogo 1996). Throughout most of its distribution, the species has two generations emerging in a summer season (bivoltine), with the first generation emerging from pupation in November to December and the second in February to March. In more arid areas, however, there is usually only one generation (univoltine). The first generation population is also considerably larger than the second one (Ditlhogo 1996), possibly due to the first generation feeding on more nutritional young leaves (Klok & Chown 1999).

Adult moths have rudimentary mouth parts and do not feed, harvesting all their water and nutrient resources while they are caterpillars (Holm & de Villiers 1983). They live for two to three days, during which time their only function is to find receptive mates and to oviposit. Female moths lay a single cluster of 30-335 eggs on the upper or lower surface of leaves (Fig. 2.6a) and on twigs or branches of mopane trees (Ditlhogo 1996; personal observation). The larvae emerge after approximately ten days and pass through five instars before pupation (Klok & Chown 1999). The larval stage lasts approximately six weeks, during which time the caterpillars' body mass increases by about 4000 fold and the fifth instars reach a length of about 80 mm (Gaston *et al.* 1997; Fig. 2.6b). At the end of the larval stage, the fifth instar climbs down to the ground and burrows into the soil, where it pupates. Depending on the generation, eclosion occurs either six to seven months after pupation.

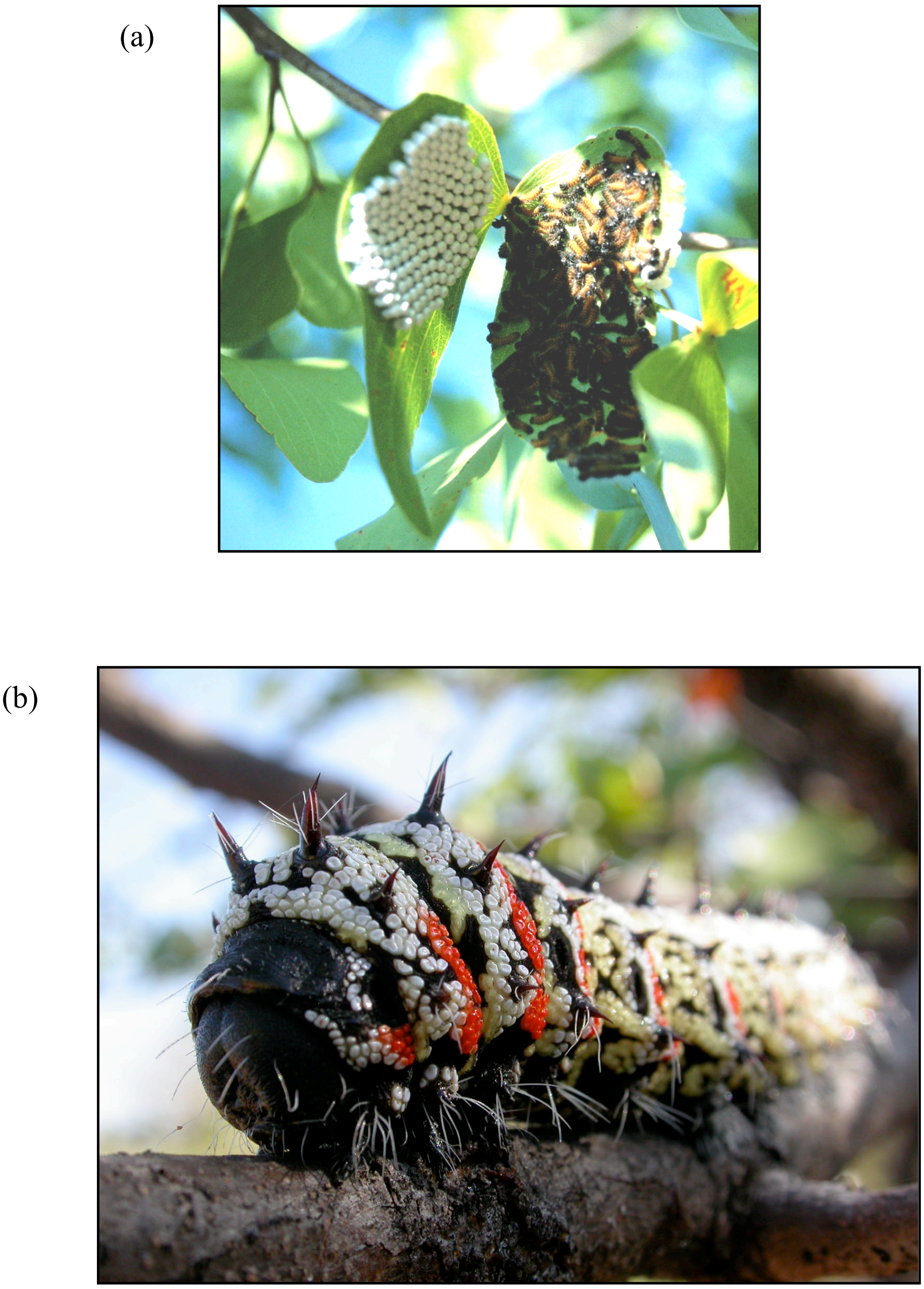

Figure 2.6 (a) An egg mass and newly hatched mopane caterpillars on a mopane leaf, and (b) a fully grown mopane caterpillar.

Mopane caterpillars are well known for their population outbreaks, which may result in large stands of trees being completely defoliated. Population numbers may vary quite considerably from year to year in any single locality however, and the cause of these variations in time and space is not yet properly understood. Factors proposed to be important in the population dynamics of mopane caterpillars include browse quality, soil type and rainfall, but recent attention has focused on the role of disease (Dwyer *et al*. 2000) and parasitoids (predominantly *Mesocomys pulchriceps*; van den Berg 1971; Berryman 1996; Maron & Harrison 1997).

The overall abundance of mopane caterpillars, which is apparently declining (Roberts 1998), may also be affected by an increase in harvesting by people, as moths are reported to have disappeared from parts of Botswana after heavy harvesting (Bartlett 1996). In addition to over-harvesting, suggested threats to mopane caterpillar abundance include deforestation of mopane woodland and increasing drought. Deforestation may be caused by the felling of trees to facilitate harvesting of the caterpillar, or through heavy elephant utilization (Styles & Skinner 1996).

Apart from humans, *I. belina* provide food for numerous other predators. Pupae are readily dug up by jackals (*Canis mesmelas*), bat-eared foxes (*Otocyon megalotis*), warthogs (*Phacochoerus aethiopicus*) and aardvarks (*Orycteropus afer*); larvae are eaten by mammals (mainly baboons, *Papio ursinus* and vervet monkeys, *Cercopithecus aethiops*), birds (34 species were documented by Styles 1995), and invertebrates (e.g. spiders, ants, and mantids; Styles & Skinner 1996); and moths are taken by birds, spiders and bats (Hill & Smith 1984).

2.4 African elephants in mopane woodlands

Mopane woodland is found extensively within conservation areas in southern Africa and within these areas, mopane often forms the principle food source in the diet of African elephants (*Loxodonta africana*, order: Proboscidea, family: elephantidae; De Villiers *et al.* 1991; Lewis 1991). Despite its abundance, mopane is selected for by elephants (Viljoen 1989; Smallie & O'Connor 2000), thereby making these woodlands susceptible to elephant induced damage (Ben-Shahar 1993).

Elephants utilise mainly branches, foliage and the main stem of mopane trees, but the type of utilisation is dependant on tree height (Smallie & O' Connor 2000). Most branch, foliage and stem utilisation occurs on trees < 4 m high and most bark utilisation on trees > 4 m in height. Pushing over of mopane trees is very rare. Elephants also tend to prefer branches from a relatively low height (< 1 m, Smallie & O'Connor 2000; 1-2 m, Caughley 1976) and therefore utilise more biomass of shrub mopane than of mature trees (Ben-Shahar 1993). Additionally, previously damaged trees of < 2 m in height are preferred to unutilised trees, as the damage-induced coppicing provides a greater availability of their preferred stem size. Consequently, elephants tend to have a greater impact on smaller mopane trees, thereby preventing the recruitment of taller trees. In northern Botswana, for example, Ben-Shahar (1998) found that even though 43% of mopane trees were affected by elephants, the density of large trees remained largely unchanged. As a result, the woodland structure is often double-tiered, comprised of coppicing shrubs and tall trees (Timberlake 1995).

While individual trees are generally only lightly utilised, breakage of the main stem can result in a 51-75% loss of biomass (Smallie & O'Connor 2000). When smaller size classes are depleted (e.g. due to drought or concentrated feeding around water holes), larger trees are also utilised more than normal, and new elephant damage to mopane increases significantly with increasing elephant densities (Ben-Shahar 1996a). Elephants can therefore have a significant impact on mopane woodland structure, sometimes reducing woodland to shrubland (Ben-Shahar 1998). However, due to the type of damage inflicted on the trees (predominantly branch and stem breakage rather than the pushing over of trees), elephant browsing tends to have little impact on tree density, even when elephant densities are high. On a regional scale, there is also no evidence that elephants reduce biomass of mopane woodlands below a sustainable level. Ben-Shahar (1996a), for example, showed that in the absence of fire, mopane woodland would only begin to decline if elephant densities exceeded 10.5 animals/km^2. This is considerably higher than current mean elephant densities within mopane woodland (e.g. 1.1 elephants/km^2 in Luangwa Valley, Zambia; Lewis 1991), but is comparable to localised densities around water sources at the end of the dry season (7 –10 animals/km^2 in northern Botswana; Ben-Shahar 1996b).

While the modification of mopane woodland structure tends to have little impact on the elephant population, it can, however, have either a positive or a negative effect on many associated species, such as birds, insects and other mammals (Cumming *et al*. 1997; Mills & Hes 1997). At high densities, they can therefore have a significant impact on the dynamics and functioning of systems, and are hence considered as keystone species in the ecology of mopane woodlands (Timberlake 1995).

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CHAPTER 3

DIFFERENTIAL EFFECTS OF DEFOLIATION BY MOPANE CATERPILLARS AND PRUNING BY AFRICAN ELEPHANTS ON THE REGROWTH OF *COLOPHOSPERMUM MOPANE* FOLIAGE

3.1 Introduction

Plant responses to herbivory depend on the timing, type and extent of damage, the availability of resources in the environment to support regrowth, and the herbivory history of the plant (Maschinski & Whitham 1989; Paige 1992; Hawkes & Sullivan 2001; Wise & Abrahamson 2005). All responses are attempts by plant species to maximise occupancy of as many sites in the ecosystem with as much biomass as possible for as much time as possible and to achieve this, trade-offs in investments often need to be made (e.g. resprouting versus seeding; Bellingham & Sparrow 2000). In the face of herbivory, for example, trade-offs between different kinds of plant resource allocation occur, such as between resistance (the ability to reduce or prevent further herbivory) and tolerance (the ability to minimise losses in fitness following herbivory; Herms & Mattson 1992; Strauss & Agrawal 1999; Agrawal 2000; Messina *et al*. 2002). This differential allocation of resources affects shoot regrowth morphology and/or chemistry that then may, in turn, influence subsequent herbivory (Strauss 1991; Danell *et al*. 1994).

A key factor determining a plant's response to browsing is the type of damage inflicted, namely, shoot/branch/stem damage by mammalian browsers (hereafter referred to as 'pruning'), versus leaf consumption by folivorous insects (hereafter referred to as 'defoliation'). Studies have shown pruning and defoliation to affect plant responses quite differently, as pruning tends to result in an increase in the growth rate of the remaining or resprouting shoots (Messina *et al*. 2002) and the production of fewer but longer shoots with larger leaves (Danell *et al*. 1994; Duncan *et al*. 1998; Bergström *et al*. 2000; Lehtilä *et al*. 2000), while defoliation tends to result in regrowth with smaller shoots and leaves and an increase in leaf density (Gadd *et al*. 2001; Anttonen *et al*. 2002; Piene *et al*. 2002). Additionally, changes in foliar chemical composition may occur and although some studies have shown that leaves often become less chemically defended after mammalian browsing (du Toit *et al*. 1990), in general the responses to defoliation are variable (as found by Bryant *et al*. 1991 after manual defoliation of six southern African woody species). The variability in responses therefore suggests that plants have adapted different 'strategies' with which to deal with each type of browsing, and many theories attempt to explain these responses. Proposed hypotheses are not necessarily mutually incompatible, however, as while the resource availability hypothesis (RAH) is based on the premise that regrowth is related to the quantity of nutrient reserves in the plant (Coley *et al*. 1985), for example, the sink-source hypothesis (SSH) explains how sink/source relationships determine resource allocation among organs, with sink strength determining the degree of resource accumulation (Honkanen & Haukioja 1994; Stowe *et al*. 2000).

Despite the numerous studies on plant responses to herbivory, the true comparative effect of defoliation versus pruning is, however, not yet fully understood, as even though much work has looked at the effects of intensity, frequency and timing of pruning and defoliation (Lennartsson *et al*. 1998), few studies have made comparisons within an individual tree species. Consequently, results would not vary due to damage type alone but also due to other factors, such as plant functional type (e.g. deciduous versus evergreen; Krause & Raffa 1996). By considering just a single species, we could therefore significantly improve our understanding of the differential effect each browsing type has, and *Colophospermum mopane* (commonly know as 'mopane', see details in Chapter 2) provides the opportunity to carry out such an investigation in a natural system.

This deciduous, xeric savanna woodland species dominates over vast areas of land in southern Africa and is browsed upon by two key species, namely mopane caterpillars (*Imbrasia belina*) and African elephants (*Loxodonta africana*).

Despite its chemical defences, mopane is selected by elephants and can form the dominant constituent of their diet within certain areas (Lewis 1991; Smallie & O'Connor 2000), thereby making woodlands dominated by mopane susceptible to elephant induced damage (Ben-Shahar 1993). Smallie and O'Connor (2000) found that for shrub-height trees, the preferred tree size by elephants, utilization was mainly of branches, foliage and the main stem, while bark utilization was mainly from trees > 4 m high. Elephants therefore have a pruning effect on mopane trees and even though, on average, individual mopane trees are only lightly utilized, stem breakage can result in the loss of 51 –75% of above-ground biomass, and the occurrence of such utilization is known to increase under conditions of limited resources (caused by drought, high elephant densities etc.). Additionally, elephants have a preference for trees previously utilised by them, which have a greater availability of the preferred branch size due to damage-induced coppicing (Smallie & O'Connor 2000). The breakage of branches and stems, together with the continuous browsing of the same trees (hedging), can therefore have a significant impact on mopane trees.

Similarly, mopane caterpillars are well known for their impact on mopane trees, as they tend to experience population outbreaks within mopane woodland, resulting in large stands of trees being completely defoliated. Additionally, while population numbers may vary quite considerably from year to year, under favourable conditions (sufficient rainfall etc.) two brood sessions can take place within one wet season, firstly in November/December and again in February/March. Trees may hence undergo complete defoliation twice within a single growing season and this accumulative effect of defoliation events (Strauss 1991; Kaitaniemi *et al*. 1999), together with the differential timing of leaf herbivory (Tiffin 2002), is expected to have a significant effect on mopane regrowth responses.

Apart from an improved understanding of plant-herbivore interactions, determining the differential influence of defoliation and pruning on mopane regrowth responses will also enable an investigation into how this megaherbivore and insect may interact through their shared resource, a novel approach for intraguild studies. The aim of this study was therefore to investigate the comparative effects of pruning by elephants and defoliation by mopane caterpillars on mopane trees (i.e. the effect of the different nature and timing of damage), by addressing the following main questions: (1) Are there quantitative differences (structural and chemical) in the regrowth of mopane following caterpillar defoliation versus elephant pruning? (2) Does timing of caterpillar defoliation (early versus late season) affect regrowth? (3) Does the frequency of caterpillar defoliation (once versus twice) affect regrowth? (4) Does the first flush of regrowth after caterpillar defoliation and elephant pruning differ to the second flush i.e. the following season?

3.2 Methods

The study was initiated in August 2002 and continued until March 2004 in the Venetia-Limpopo Nature Reserve (refer to Chapter 2 for details).

3.2.1 Treatment transects

Six 80 m transects were set out within a flat and uniform area of mopane woodland, with a mean tree height of 2.17 m (sd. = 0.637, n = 144). Three transects were on each side of the road, positioned parallel, perpendicular and at 45° to the road. Along each transect, a tree was marked every 20 m with a metal tag nailed into its base (5 trees per transect i.e. n = 30). Around each marked tree, six additional trees, positioned roughly 60° apart and 2 – 8 m away from the central tree, were marked. Trees of similar size and with minimal elephant utilization were chosen. This orderly layout of experimental trees was necessary to ensure they could be found again in the dense, homogenous mopane woodland.

To test the comparability of transects, soil samples were collected from five points along each transect (i.e. at each tree group). Samples were taken from the top 10 cm of soil and air-dried in brown paper bags. Analyses were carried out to determine the water pH and soil P, Ca, K, Mg, Na and N content (according to the methodology stated by The Non-Affiliated Soil Analysis Work Committee 1990). Mineral results were expressed in mg/kg.

In August 2002, at the time of year when utilization of woody plant parts by elephants is highest (Lewis 1986), one tree from each tree group was subjected to simulated elephant browsing. Stems and branches were broken off at 1 – 1.5 m (the preferred browsing height of elephants; Smallie & O'Connor 2000), until about 75% of the original biomass was removed (estimated visually). In August 2003 the same treatment was applied to a second tree in each group.

To simulate the two successive mopane caterpillar defoliation events, one tree in each group was artificially defoliated in November 2002; one in February 2003; one in November 2002 and again in February 2003 and one in November 2003. Trees were at least 90% defoliated, by removing the entire leaf lamina by hand while taking care to leave behind the leaf petiole, to best mimic true caterpillar defoliation. The seventh tree in each group was left as a control. Treatments are hereafter referred to as follows: (1) Elephant 2002 = E'02, (2) Elephant 2003 = E'03, (3) Caterpillar November 2002 =

CN’02, (4) Caterpillar February 2003 = CF’03, (5) Caterpillar November 2002 and February 2003 = CN&F, (6) Caterpillar November 2003 = CN’03 and (7) Control = Con.

The validity of using simulated herbivory to mimic natural damage has been questioned in recent years (Baldwin 1990; Tiffin & Inouye 2000). Hjältén (2004) summarises the problems and possibilities of simulated herbivory and concludes, however, that mechanical damage is acceptable in the following circumstances: (1) when used to evaluate the direct effects of herbivory on basic plant processes such as growth, reproduction and general physiological responses (e.g. plant allocation to secondary compounds and changes in nutrient concentrations); and (2) for insect herbivores that inflict well-defined damage to plants that is easy to mimic with accuracy (e.g. external leaf-feeding insects). This study fulfils both these requirements and simulated herbivory was therefore expected to provide reliable results. Additionally, simulated herbivory has a number of advantages over natural herbivory, namely: the ability to control the magnitude and timing of damage, and avoiding any bias resulting from biotic and abiotic affects.

There are, however, still three main problems with simulated herbivory that may influence the results of this study. First, is the length of time over which the damage is inflicted. Mechanical defoliation took a maximum of two hours per tree, while naturally it would be a continuous process over about three weeks. Secondly, some insect herbivores have been found to alter plant physiology by injecting salival components while feeding (Alborn & Turlings 1997; McCloud & Baldwin 1997). This has also been found for mammalian browsers, such as moose (Bergman 2002) and goats (Rooke 2003). Lastly, urine and faeces of mammals and insects can have a strong effect on soil processes (nutrient recycling), which can then influence plant communities (Hobbs 1996; Reynolds & Hunter 2001).

3.2.2 Natural mopane caterpillar and elephant browsing

To check the reliability of the simulation results, for reasons outlined above, the effects of natural elephant and mopane caterpillar browsing were also assessed. In January 2004, when an outbreak of mopane caterpillars had just taken place, three transects were set out within defoliated areas. Along each transect, five defoliated trees were marked and for each of these, the nearest non-defoliated mopane tree of similar size was marked as the trees' partner (total n = 15 for each 'utilization'). An area with a high degree of elephant utilization was also identified and 13 elephant-utilized trees were marked, along with 13 non-utilized 'partners'.

3.2.3 Measuring regrowth

In November 2003, an outbreak of puss moth caterpillars (Family: Notodontidae, Order: *Epicerura*; Picker *et al*. 2002) defoliated most trees along three of the six transects. These transects were therefore excluded from the experiment, reducing the sample size of each treatment to 15 trees.

In February 2004 trees were revisited and the following information on the regrowth was recorded: leaf size, shoot length, shoot density and foliar nutritional value. Leaf length was used as an indicator of leaf size (as leaf length and width are correlated, see Appendix A), measured (in mm) from the growth point to the tip of the right leaflet. Fifteen mature undamaged leaves were measured for each tree and were chosen randomly from all sides of the canopy, between a height of 0.5 – 2 m. The length of the current years growth (which was clearly identifiable) was measured for 15 shoots per tree (to the nearest cm), chosen randomly from pruned and unpruned parts of the tree. Shoot density was measured as the number of shoots on the terminal 50 cm of a branch, for five branches per tree.

To determine foliar chemical composition, mature leaves were collected randomly from around the canopy of each tree. A detailed chemical analysis of secondary metabolites was not in the scope of this project, especially seeing as mopane is well known for its complex chemical make-up (Ferreira *et al*. 2003). Secondary metabolites such as tannin, that accumulate in high concentrations and are often stable end products, are most likely to be directly correlated with total allocation to secondary metabolites (Herms & Mattson 1992). Total polyphenolic and condensed tannin content were therefore determined, together with the protein (N) content.

Leaves were first air dried in brown paper bags and later oven dried at 50°C for at least four days. Samples were then milled through a 1mm screen. The crude protein content was determined according to an AOAC (2000) method, namely the Dumas method. Total phenols and condensed tannins were analysed in accordance with Hagerman (1995). Total phenols were quantified using the Prussian blue assay for total phenols and condensed tannins were assayed using the Acid butanol method for proanthocyanidins. Dried leaf material weighing about 0.05 g was extracted with 3 ml of 70% aqueous acetone by sonicating in an ice-water bath for 30 min and centrifuging at 2000 g for 10 min. A 0.1 ml aliquot of the supernatant was used in both assays. *Sorghum* tannin that was previously extracted and purified as described in Hagerman (1995) was used as the condensed tannin standard while Gallic acid was used to standardize total phenols. Quantities were calculated as mg/gDW (dry weight).

I appreciate that chemical composition values obtained in this study are not absolute leaf concentrations, as these were not possible to obtain with the field methods used. Samples should ideally have been freeze dried in dry ice soon after collection instead of being air dried, for example, but this was not possible due to the long period of time spent in the field. Values were, however, used only as relative measures for comparative purposes within the study, and are therefore suitable for this purpose.

To investigate the effect of treatment on seasonal leaf carriage, trees were inspected in June and August 2003 for leaf retention into the dry season and in October and November 2003 for the timing of leaf flush. Trees were given a leaf carriage score (LCS) where: LCS = 0 means no leaves, LCS = 1 means 1-10% of full leaf carriage, LCS = 2 means 11-40% of full carriage, LCS = 3 means 41-70% of full leaf carriage, and LCS = 4 means 71-100% of full leaf carriage. This leaf score was then subdivided into the phenological states of the leaves, using the same scoring system (Smit 1994). Leaf phenological states were classified as: (i) newly formed leaf buds (LB), (ii) immature green leaves (IL), (iii) mature green leaves (ML), (iv) yellowing, senescing leaves (YL), and (v) dry, senescing leaves retained on the tree (DL). For example, a tree could have a total LCS = 3, consisting of 80% IL (LCS = 4) and 20% ML (LCS = 2).

In March 2004, once the regrowth on the naturally defoliated trees had stopped growing (see Appendix B, Fig. B.1), all control and utilised trees (elephant and caterpillar) were revisited and information on the current years growth recorded. Leaf size, shoot length and shoot density were measured as described above. Leaf samples were also collected for chemical analysis.

3.2.4 Statistical analysis

For simulated treatments, I first tested whether there was significant variation in the regrowth (shoot length, leaf length, shoot number/50cm, and foliar tannin: protein ratio and polyphenolic content) and soil characteristics across the three transects, using one-way ANOVAs and Kruskal-Wallis ANOVAs. Then, due to the data not being normally distributed, Kruskal-Wallis ANOVAs were used to test for differences between the seven treatments, for each variable individually. Where results were significant, multiple comparison tests were used to determine which treatments were different.

Data from the naturally utilised trees were also not normally distributed, so Wilcoxon matched-pairs tests were used to test for differences between the regrowth on elephant-utilized trees and control trees, and between caterpillar defoliated trees and control trees (Zar 1999). All statistical analyses were conducted using STATISTICA (Statsoft 1991).

3.3 Results

3.3.1 Simulated treatments

There was no significant variation across the three treatment transects with respect to any of the regrowth characteristics or soil properties, except the soil Mg content (see Appendix C for soil details). It was therefore assumed that no 'transect' effect was influencing the regrowth of the trees and trees from all three transects were then grouped for analyses.

Simulated treatments had a significant effect on shoot length ($\chi^2 = 42.0$, df = 6, $P < 0.01$) and leaf length ($\chi^2 = 28.4$, df = 6, $P < 0.01$). Shoots were generally longer on pruned trees than defoliated trees (Fig. 3.1a). Trees flushing for the first time since pruning (E'03 trees) produced shoots significantly longer (mean ± SE: 15.8 ± 0.693 cm) than pruned trees flushing for the second time (E'02 trees; 11.1 ± 1.04 cm), whose shoots were not significantly longer than control tree shoots (6.99 ± 0.221 cm). Shoots on most defoliated trees (but not CN'03 trees) were significantly shorter than shoots on pruned trees, but not control trees. Although not significantly different, trees defoliated in February tended to have slightly shorter shoots than trees defoliated in November (5.23 ±

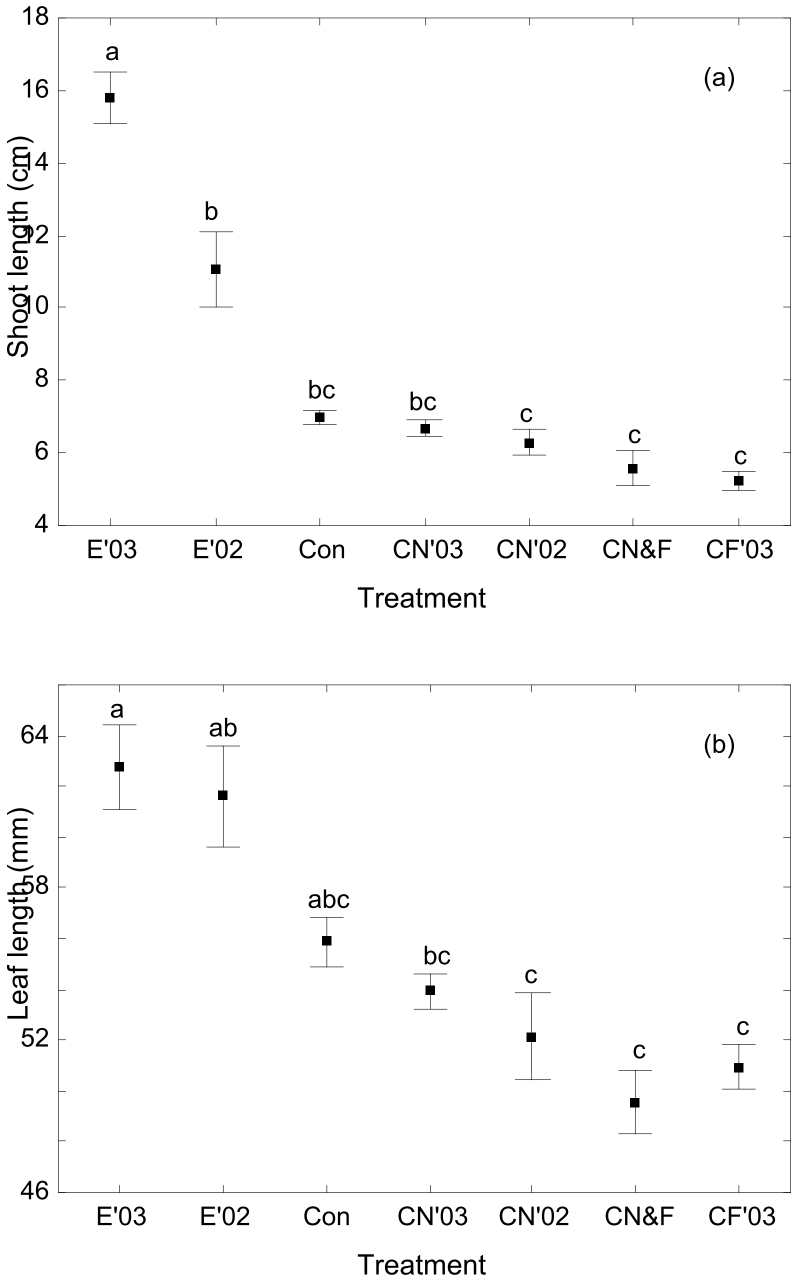

Figure 3.1 A comparison of the effects of various elephant pruning and caterpillar defoliation treatments on (a) mean (± SE) shoot length and (b) leaf size of mopane regrowth. Unlike letters (a, b and c) indicate significant differences. Treatments included: simulated elephant pruning in August 2002 (E'02) and August 2003 (E'03), controls (Con), and simulated caterpillar defoliation in November 2002 (CN'02), February 2003 (CF'03), November 2002 and February 2003 (CN&F), and November 2003 (CN'03).

0.270 and 5.57 ± 0.485 cm versus 6.30 ± 0.350 and 6.67 ± 0.223 cm). Similarly, leaves on pruned trees were significantly longer than leaves on defoliated trees, except for CN'03 trees (Fig. 3.1b, pruned: E'03 = 62.8 ± 1.67 and E'02 = 61.6 ± 1.98 mm versus defoliated: CN'02 = 52.1 ± 1.70, CN&F = 49.5 C 1.27 and CF'03 = 50.9 ± 0.875 mm). As with shoot length, leaves on trees defoliated in February tended to be smallest. Late season defoliation therefore appears to have the greatest negative impact on regrowth in mopane.

Shoot number did tend to be greater after defoliation treatments than pruning treatments (χ^2 = 12.2, df = 6, *P* = 0.057; the two extreme measures being: CN = 14.45 ± 0.67 and ET = 11.60 ± 0.48) and even though this difference was not quite significant at the 5% level, it is still considered a real trend (see Stewart-Oaten 1995). There was, however, no difference in the tannin:protein ratio or total polyphenolic content of the foliage (χ^2 = 3.59, df = 6, *P* = 0.732; χ^2 = 2.00, df = 6, *P* = 0.92 respectively).

Browsing treatment appeared to affect leaf carriage in a number of ways. Firstly, trees defoliated in February had a very low total leaf carriage in June and August (Fig. 3.2), indicating they were unable to flush again before the onset of the dry season. Pruned trees, however, tended to have a greater leaf carriage later into the dry season (August), suggesting that pruning aids leaf retention in remaining foliage. Secondly, all defoliation treatments resulted in trees producing leaf buds earlier in the growing season than control and pruned trees (October). By November, trees from all treatments had flushed nearly 100%, with roughly half of the buds already having grown into immature leaves.

3.3.2 Naturally browsed trees

Regrowth on trees naturally damaged by elephants was similar to the simulated treatment trees. Shoots and leaves were significantly longer than on control trees (Wilcoxon

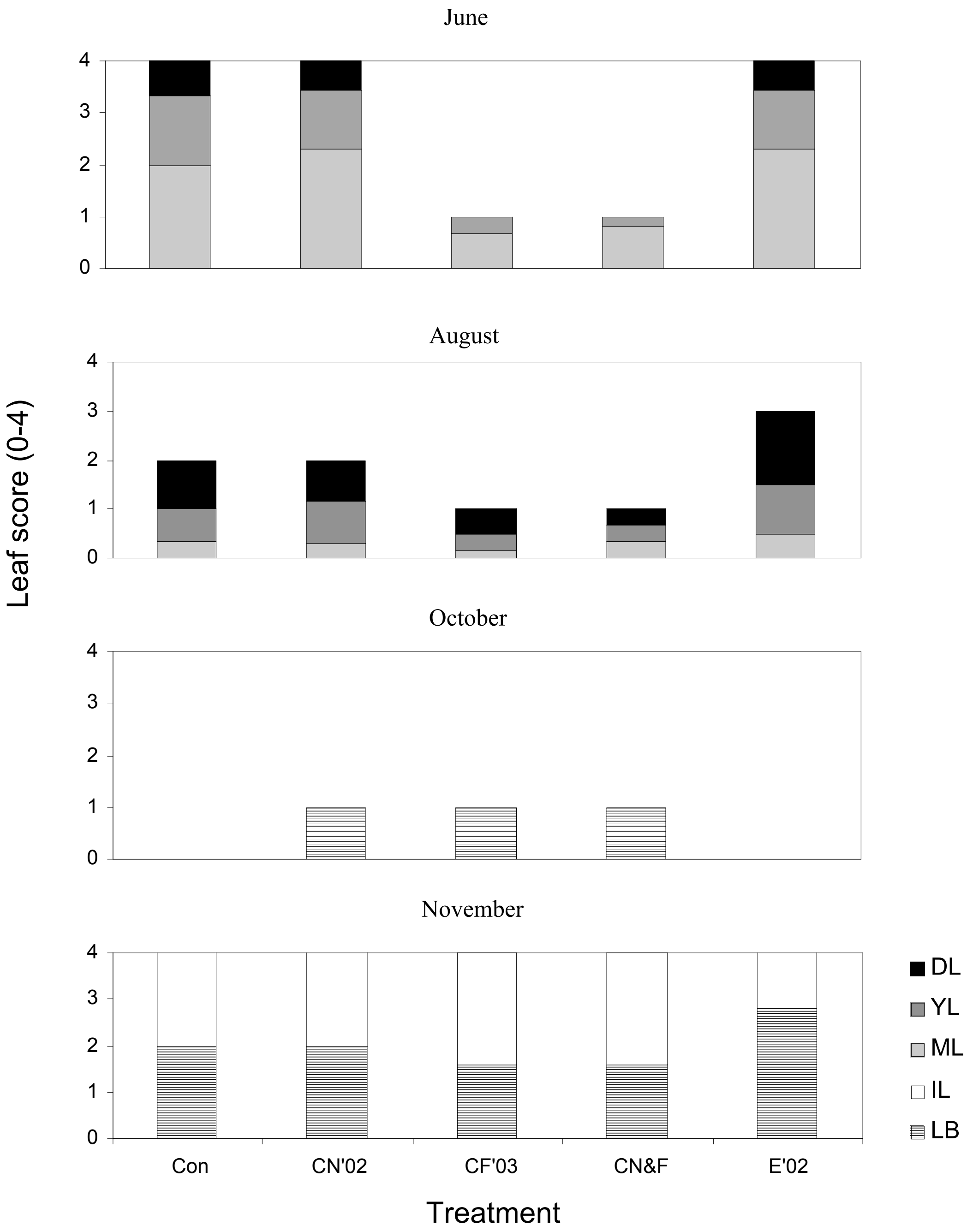

Figure 3.2 Seasonal leaf carriage of mopane trees after simulated caterpillar and elephant utilization treatments (refer to in Fig. 3.1 for treatment code details and the text for score details). DL = dead leaves, YL = yellow leaves, ML = mature leaves, IL = immature leaves, LB = leaf buds.

matched-pairs test: *P* < 0.01; shoots: 18.2 ± 1.34 versus 7.03 ± 0.341 cm; leaves: 62.6 ± 1.65 versus 49.6 ± 1.07 mm), while there was no difference in the number of shoots/50 cm length of branch (*P* = 0.859), nor in the tannin:protein ratio and total polyphenolic content of the foliage (*P* = 0.507, *P* = 0.249 respectively; Fig. 3.3).

Natural caterpillar defoliation, however, had a greater effect on plant responses than simulated defoliation. Both shoot and leaf lengths were significantly shorter on previously defoliated trees than control trees (Wilcoxon matched-pairs test: *P* < 0.01; shoots: 3.70 ± 0.135 versus 7.15 ± 0.231 cm; leaves: 45.4 ± 1.36 versus 57.3 ± 1.79 mm) and foliar tannin:protein ratio and total polyphenolic content were significantly reduced (tannin:protein ratio: 0.348 ± 0.027 versus 0.443 ± 0.027; polyphenols: 52.4 ± 3.67 versus 63.9 ± 3.39 mg/gDW; Wilcoxon matched-pairs test: *P* < 0.05; Fig. 3.3). Only shoot density was unaffected (*P* = 0.211).

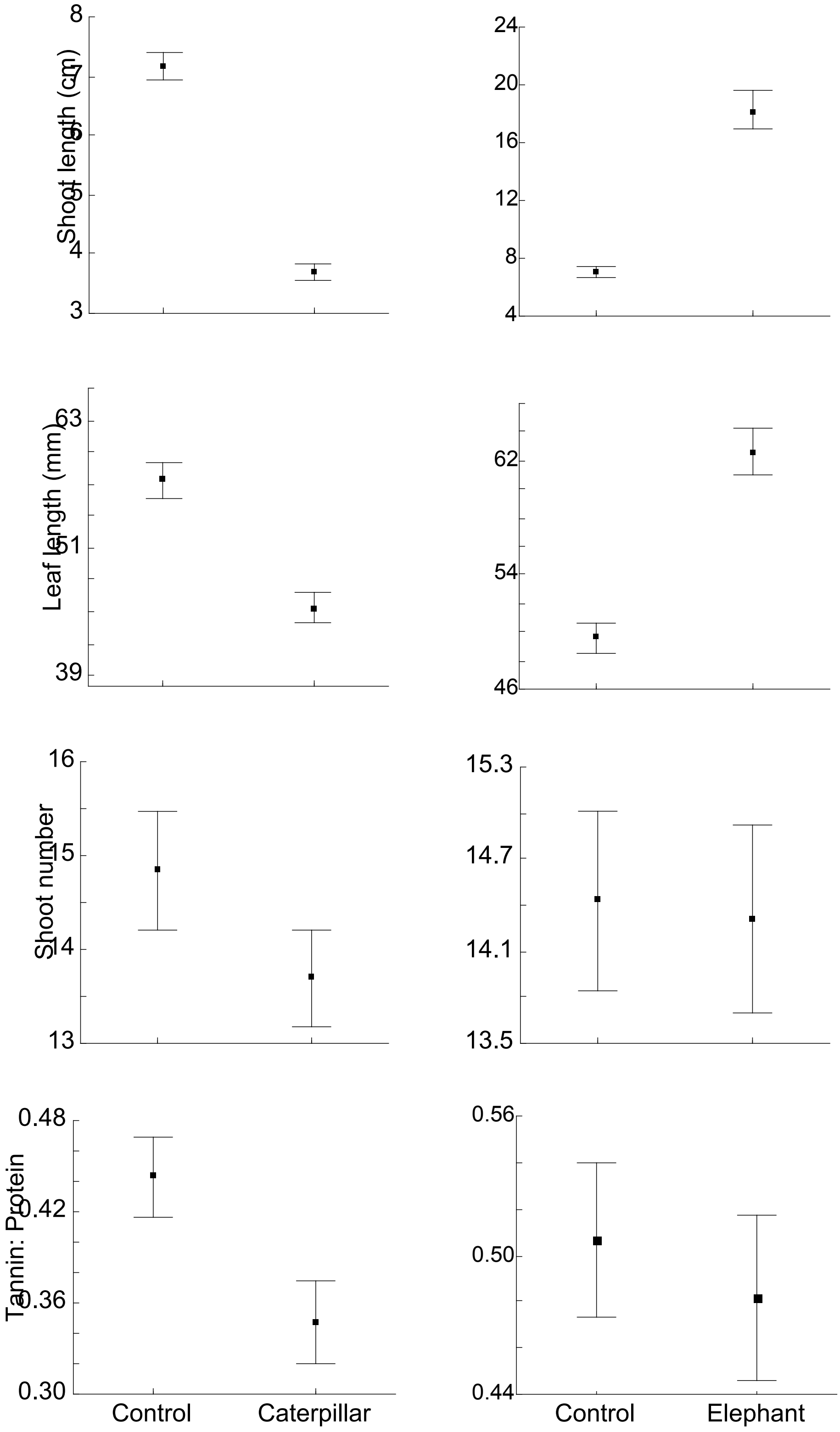

Figure 3.3 The effect of natural elephant pruning and mopane caterpillar defoliation on the regrowth of mopane trees (mean ± SE values).

3.4 Discussion

Both mopane caterpillars and elephants cause extensive damage to individual mopane trees, yet the two types of herbivory affect plant responses in significantly different ways. Pruning by elephants resulted in the production of longer shoots and leaves, while defoliation by caterpillars had the opposite effect. Foliar chemical composition was only found to change after natural caterpillar defoliation, where an increase in nutritional value occurred. The difference in plant responses found here supports results from numerous other studies on browsing (e.g. Bryant *et al*. 1991; Danell *et al*. 1994; Lehtilä *et al*. 2000), and results are as expected considering the differential affect each damage type has on resource availability and resource allocation within individual plants.

According to the sink/source hypothesis (SSH), for example, damage affects plant growth primarily by changing the ability of meristems to compete for resources (Honkanen & Haukioja 1994). In undamaged individuals, sink-source relationships determine resource allocation among organs, with sink strength determining the degree of resource accumulation. Damage through browsing often removes sinks and/or sources, however, thereby altering relationships and modifying allocation patterns (Stowe *et al*. 2000). Because defoliation and pruning affect functionally different plant tissues (physiological sources or sinks), it is hence not surprising that they have variable effects on growth. Defoliation, for example, weakens sink strength of meristems formed immediately after damage, thereby leading to poor plant growth, while branch/stem removal destroys apical meristem dominance (strong physiological sinks) of entire branches, thereby resulting in the redirection of resources towards lateral meristems, which would otherwise remain dormant.

While changes in sink/source relationships explains how damage could affect the distribution of growth activity, the overall regrowth of a plant is also dependent upon the quantity of nutrient reserves in the plant (i.e. resource availability). During summer, photoassimilates are accumulated and transferred to stems and roots, where they are stored for growth the following spring. Removal of storage organs reduces these carbon and mineral reserves, thereby reducing overall plant growth (Quiring & McKinnon 1999). In mopane woodland, the timing of the main defoliation event (November/December) is such that plants would have utilized stored resources for new shoot production (i.e. acting as sinks), but would most likely not have had sufficient time to replenish the used reserves through photosynthesis. Leaf removal at this time would therefore result in an overall decrease in resources available compared to at the start of the first flush. Enhanced by the increase in shoot number after defoliation, less resources are then available per shoot, resulting in a decrease in shoot and leaf size. A similar response has been observed for other southern African deciduous species, such as *Acacia tortilis*, *Grewia flavescens and Dichrostachys cinerea* (Bryant *et al*. 1991). The response to elephant utilization, which was opposite to defoliation, is also as expected considering the probable changes in resource availability within the plant. The high intensity of branch/stem breakage had a significant impact on the root/shoot ratio, as up to 75% of the canopy biomass was removed. When part of the photosynthetic material of a plant is removed, potentially more water and nutrients are then available for the remaining photosynthetic material, resulting in increased shoot and leaf growth (Alados *et al*. 1997). Similarly, the shorter shoot length on trees flushing for the second time (E’02) compared to those flushing for the first time (E’03) can be explained in this way, as the root/shoot ratio would have decreased after the first flush.

In accordance with results from other studies is the greater negative impact of late season (February) defoliation on regrowth found here (Maschinski & Whitham 1989; Danell *et al*. 1994). According to Tiffin (2002), early and late-season damage is more detrimental than mid-season herbivore damage for various reasons. During early season defoliation, leaves may be removed while still growing and acting as sinks, and are therefore removed before having a chance to act as sources and replenish resources used for growth. Similarly, if defoliation takes place too late in the growing season, time for regrowth and replacement of lost resources before the dry season may be insufficient (Maschinski & Whitham 1989; Lennartsson *et al*. 1998). Consequently, growth the following season is retarded. This explains the reduced growth after February (i.e. late-season) defoliation here, as very few mopane trees were observed being able to flush again before the dry season, while November-defoliated (i.e. mid-season) trees re-flushed readily. It should be noted, however, that the summer during which late-season treatments were applied (February 2003) was hot with little rainfall after February (see Fig. 2.3), resulting in a very poor mopane caterpillar crop (most died of desiccation) and minimal defoliation of trees. The following season (2003/2004) experienced good rainfall into March, however, and stands of trees were observed to re-flush each time after three complete defoliation events (once was by puss moth larva). It may therefore have been an unusual situation for mopane trees to incur defoliation and reduced resource availability simultaneously, as in more arid areas there is naturally only one generation of caterpillars (Oberprieler 1995). Growth of mopane is known to be dependant upon water availability, as nitrogen mineralistaion requires the soil to be moist (Henning & White 1974). The already short period for regrowth and nutrient replenishment after the February defoliation would then have been made even worse by the low late-season rainfall in 2003.

While the differential response of mopane trees to pruning and defoliation is consistent with current theory, it is interesting to consider how these responses may help mopane trees withstand the pressures of herbivory. The degree to which a plant is able to respond to and tolerate herbivory depends upon both the extent of damage inflicted and the amount of resources stored belowground. The interaction between resource allocation and resource availability is therefore important, as a plants ‘decision’ about how to invest it’s accumulated resources will directly influence the amount of stored resources available for regrowth after damage (Stowe *et al*. 2000). Various factors are known to influence the size of storage organs, such as the interaction between the long-term frequency and severity of disturbance (Riba 1998), as well as site productivity (Bellingham & Sparrow 2000). In areas of low frequency disturbance, for example, the production of storage organs would not be worthwhile, as it would just constrain aboveground growth and reduce the plants’ competitive ability. Site productivity can influence whether a plant invests in resprouting or seeding and, according to Bellingham and Sparrow (2000), more fertile and moist sites tend to promote seed production as compared to dry and less fertile sites. Mopane woodland, which tends to occur on resource limited sites (see review by Timberlake 1995), also experiences relatively frequent and severe disturbance events, in the form of defoliation or drought. It is therefore not surprising that this species has an exceptionally high root biomass (Smit & Rethman 1998), which allows it to regrow rapidly (Cheplick & Chui 2001). Interestingly, this high investment in root material by mopane may actually explain the decrease in leaf size after defoliation, as Papatheodorou *et al.* (1998) found that with increased browsing of *Quercus coccifera* shrubs, coarse root material increased but leaf size decreased. Mopane trees may therefore also have adapted this precautionary approach of increasing resource allocation below ground in response to browsing.

Additional factors that could help in mopane’s tolerance to browsing are leaf life span and leaf size. Extended leaf carriage period, together with increased leaf size after pruning, for example, allow for more photoassimilates to be accumulated and resources are therefore better replenished (Nowak & Caldwell 1984; Stowe *et al*. 2000). The increase in leaf life span after elephant utilization can be explained in terms of the RAH, as not only is the plants' water status improved, but pruning can also reduce the competition between leaves for root-derived cytokinins, which have been found to delay leaf senescence (Salisbury & Ross 1985). Additionally, although not measured, an apparent increase in leaf thickness may also explain the increase in leaf life span, as the two are often positively correlated (Wright *et al*. 2002).

Defoliation did not affect leaf senescence (due to there being no change in competition between leaves), yet the nutritional value of leaves was significantly improved after natural defoliation. This could also help in the replenishment of resources, as a higher N content in leaves increases a plants' photosynthetic capacity, thereby acting as a compensatory response to the loss in leaf area (Kazda *et al*. 2004). Additionally, an apparent reduction in leaf thickness (although not measured) after defoliation would further enhance the rate of increase in photosynthetic capacity, as light intensity decreases with depth from the leaf surface (Hattori *et al*. 2004). Therefore, just as increased leaf size and life span potentially improved mopane tolerance to pruning (by increasing the rate of resource replenishment), decreases in chemical defences and leaf thickness may enhance mopane tolerance to defoliation.

Interestingly, unlike natural defoliation, no change in leaf chemistry was recorded after simulated defoliation. The apparent improvement after natural defoliation could be due to the younger leaf age (Styles & Skinner 1997), yet leaves from the regrowth of trees manually defoliated in November 2003 were also younger than from control trees, but were no more nutritional. Additionally, the difference in shoot and leaf length was notably greater after natural versus simulated defoliation, indicating natural defoliation did have a genuinely greater impact. One explanation for differences in response could be that simulated herbivory did not sufficiently mimic natural defoliation (in various ways discussed earlier). Alternatively, differences could be due to variation in individual tree history. During the study period, it was observed that mopane caterpillar abundance was very low within the area in which treatment trees were situated. Previous defoliation of treatment trees was therefore probably minimal. Naturally defoliated trees, however, were frequently observed to have a high abundance of mopane caterpillars and were frequently defoliated. The greater impact recorded on these trees may therefore be due to a long-term accumulative effect of repeated damage, such as that found for *Erica arborea* (Riba 1998). Furthermore, there may already have been differences between trees selected for oviposition by moths or browsing by elephants and the unselected partner trees, resulting in measured responses not only reflecting the effect of pruning and defoliation, but also initial differences between pairs.

The decrease in resistance with an increase in mopane caterpillar damage is unusual, as it is generally accepted that a co-evolutionary relationship exists between a plant and its primary pest (a specialist herbivore), with the frequency of resistance alleles being highest in those populations in which the pest is most common (Purrington 2000). When considering the conditions under which mopane grows, however, the negative relationship found here is not surprising, as the optimal allocation of resources (in this case defence versus growth) depends on the frequency and severity of attack (Doak 1992; Messina *et al*. 2002), both of which mopane tend to experience to a high degree. Additionally, host preference by mopane moths does not appear to be influenced by leaf nutritional value (see Chapters 5 & 6), in which case increases in chemical defences would not act as a deterrent and increased investment in costly chemical defences (Coley 1988; Bergelson & Purrington 1996; Jones & Hartley 1999; Strauss *et al*. 2002) would not be worthwhile, as the chance of further defoliation would remain high and the cost of leaf loss would be even greater. Instead, when resources are invested in growth, this helps replenish stores through increased photosynthesis (Caldwell *et al.* 1981; van der Meijden *et al*. 1988).

In conclusion, mopane trees respond differently to pruning and defoliation due to changes in sink/source relationships and resource availability. Even though no conclusions are made about the overall effect on plant fitness in this study (as this was not measured), defoliation and pruning were found to have a negative and positive effect on regrowth respectively. Time since damage (i.e. first versus second flush) also had a significant influence on regrowth after pruning, as shoot and leaf length were significantly longer on trees flushing for the first time, and within-season timing of damage was important for defoliation, as late-season defoliation had a greater negative impact than mid-season defoliation. Despite the differences in regrowth responses after defoliation and pruning, both types, however, promote the replenishment of stored resources and therefore both enhance the plant's tolerance to herbivory. This, together with the prior pattern of resource allocation to storage organs, indicates how mopane trees are adapted to deal with the impacts of herbivory, of either form.

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CHAPTER 4

EFFECTS OF PRUNING BY ELEPHANTS AND DEFOLIATION BY MOPANE CATERPILLARS ON REPRODUCTION IN *COLOPHOSPERMUM MOPANE*

4.1 Introduction

Herbivory is known to have a significant impact on plant fecundity, but while some studies have reported an increase in flower and seed production with herbivory (Strauss 1991; Paige 1992; Escós *et al*. 1996; Alados *et al*. 1997; Lennartsson *et al*. 1998; Díaz *et al*. 2004) others have found either no effect (Meyer 2000) or a negative effect (Juenger & Bergelson 2000; Peinetti *et al*. 2001; Freeman *et al*. 2003). Factors responsible for the variability in response include: intensity, timing and frequency of herbivory (Maschinski & Whitham 1989; Marquis 1992); resource availability (Lennartsson *et al*. 1998); inter and intra-species competition (Tiffin 2002); and specific characteristics of each plant species. Similarly, the type of damage associated with herbivory is expected to have a significant influence on plant reproduction, yet this specifically has not yet been investigated.

In southern African, the tree species *Colophospermum mopane* (commonly known as 'mopane') is browsed upon by two key species that each inflict a different type of damage while feeding. Mopane caterpillars (*Imbrasia belina*) defoliate mopane trees by removing just the leaf blades (Ditlhogo 1996), while African elephants (*Loxodonta africana*) have a pruning effect by breaking off branches and/or the main stem (Lewis 1991; Smallie & O'Connor 2000). The impact that each damage type has on mopane trees is known to differ in terms of regrowth responses (Chapters 3 & 6) and while this may help the current generation's tolerance to herbivory (Strauss & Agrawal 1999), the ability to maintain reproductive output is also an important aspect of plant tolerance to consider

(Juenger & Bergelson 2000). Determining the impact of defoliation versus pruning on reproduction would therefore improve our understanding of the overall affect each browsing type has on the species' fitness.

According to the Resource Availability Hypothesis (RAH), the extensive removal of aboveground biomass results in an increased availability of resources per shoot for regrowth (Coley *et al.* 1985). This explains the increased growth of individual shoots after elephant pruning found in Chapter 3, and likewise, an increased production of flowers and seeds may also be expected on these shoots. Such a response was reported for *Gentianella campestris*, for example, where the removal of half the plant biomass by clipping significantly increased fruit production (Lennartsson *et al.* 1998). Unlike pruning however, defoliation tends to result in a decrease in resource availability per shoot, and thus a decrease in reproduction is expected after caterpillar utilization (e.g. Zvereva & Kozlov 2001).

Apart from the type of damage inflicted by each browser, the timing of elephant utilization of mopane trees is also different to that of mopane caterpillars. Pruning by elephants takes place mainly at the end of the dry season (August; Lewis 1986), while defoliation by mopane caterpillars takes place around November/December and again in February/March (during the two brood sessions). The timing of herbivory relative to the reproductive phenology of a plant can have a significant effect on reproduction, as found for the forest shrub *Piper arieianum*. When subjected to simulated defoliation three months before flowering, seed production in *P. arieianum* decreased significantly, but when defoliated at the time of flowering, no difference in seed production was recorded (Marquis 1992). Flowering in mopane trees occurs between December and March, but mainly during January and February (Smit 1994), which is just after the first defoliation event but about four months after the main period of pruning by elephants. Timing, together with damage type, may therefore be expected to influence the effect that each browser has on mopane tree reproduction.

An additional difference in browsing by the two species is the size of tree targeted. While elephants tend to prefer smaller trees of < 2 m in height (Smallie & O'Connor 2000), host preference by ovipositing mopane moths is positively related to tree height (Chapters 5 and 6). Considering the impact of herbivory is often size-specific, with the negative effect decreasing with increasing tree size (as larger trees also have a larger storage of resources available for recovery; Alados *et al.* 1997), any apparent difference in tolerance to defoliation versus pruning may then be partly due to a bias in tree size. Additionally, the onset of flowering in trees is often size related, as only trees with sufficient stores can afford to invest in reproduction. If true for mopane, trees capable of flowering (i.e. taller trees) may be utilised more by mopane caterpillars than elephants, and the effect of defoliation may then have a greater direct impact on reproduction at the community level than pruning. On the other hand, elephants can prevent the recruitment of trees into the reproductive size class, thereby having an indirect impact on the overall seed production. Tree size as a determinant of reproduction is therefore important to establish to fully understand the impact by each herbivore.

Together with seed number, the successful germination and establishment of seedlings is also an important determinant of plant fitness, and a parameter influencing seedling survival is seed mass. Larger seeds generally have a higher probability of emergence and develop into seedlings with a better competitive ability than small seeds, as they have a larger store of resources (Stanton 1984). For mopane, a previous study by Smit (1994) showed tree thinning to have a significant positive effect on seed mass (presumably because resource availability per tree was improved), yet no relationship between seed mass and germination potential was found. The effect of seed mass on seedling survival is, however, still unknown and seed size could therefore also be a possible measure of plant fitness in mopane.

Considering the various tree and pod characteristics capable of influencing a plant's reproductive success, this study set out to determine the impact that defoliation by mopane caterpillars and pruning by elephants have on mopane reproduction. The following key questions were addressed: (1) Is the leaf:pod ratio affected by previous caterpillar defoliation or elephant pruning in the same season? (2) Does pruning or defoliation affect seed mass? (3) Is the likelihood of a tree flowering affected by prior defoliation in the same season? (4) Is the occurrence of reproduction in mopane trees related to tree height?

4.2 Methods

4.2.1 Field work

The study was carried out within the Venetia Limpopo Nature Reserve, South Africa (see Chapter 2 for details). Initially, the plan was to look at the effect of defoliation and pruning on reproduction by measuring pod production on experimental trees subjected to simulated defoliation and pruning (same trees as in Chapter 3). However, none of the experimental trees or trees within the experimental area flowered. Therefore, due to the unpredictability of flowering in mopane trees, only trees already flowering were used in this study.

Twelve flowering trees that had been defoliated by mopane caterpillars in December 2003 were identified (they had not yet re-flushed) and marked in January 2004, together with 12 flowering but non-defoliated trees. In a separate area, seven flowering trees previously utilised by elephants were then marked, together with seven flowering but unutilised trees. The lower number of pruned versus defoliated trees was due to their low availability. Control and utilised trees of a visually similar size (or potential size) were chosen in each case, but live tree height (m) and stem diameters (cm) were recorded to test this quantitatively. Due to the negative effect elephant damage can have on tree height, the potential similarity in tree size for elephant utilised and unutilised trees was determined by comparing the largest stem diameter from each tree, as tree height and largest stem size are correlated in mopane (Chapter 6).

Measures of flowering were not used to assess reproduction, as the timing of flowering within and between trees is highly variable for mopane. Branches were observed to be producing pods while others were just beginning to flower for instance. For accurate comparisons, trees would therefore have had to be monitored regularly over a number of weeks, which was not feasible here. Instead, pod production was therefore recorded, as this was more stable over time.

In March 2004, once pods had developed, marked trees were revisited and their reproductive effort recorded. Firstly, on the tree level, the percentage of canopy volume made up of leaves versus pods was estimated. Secondly, the number of pods and leaves on the terminal 50 cm of a branch were counted, for five branches per tree. Branches were chosen at random from around the canopy, but only those with pods were used. This was because the lack of pods could have been due to a lack of flowers and the aim was to assess how defoliation affected pod production, where pods could potentially develop. Similarly, only trees with more than 10% pods were measured in this way, as sufficient branches with pods were required. As a result, the sample size of defoliated and non-defoliated trees was reduced to seven and pruned and non-pruned trees were reduced to five and six trees respectively.

For trees with enough accessible pods, 10 pods were collected at random from around the canopy. Pods were kept in brown paper bags and were first air-dried and then oven dried at 70°C for 5 days, until a constant mass. Each pod was then weighed, at an accuracy of 0.001 grams, after which the seed was removed from the pod and weighed separately. Some seeds had been parasitised by beetles and could not be used, as the consumption of part of the seed would have reduced the mass. However, the percentage of seeds parasitised per tree was recorded to determine if previous utilization influenced a trees vulnerability to seed parasitisation.

To investigate whether defoliation influences a trees' likelihood of flowering in the same season, an additional 40 defoliated and 40 non-defoliated trees were marked in January 2004. As before, trees of similar size were selected and their height was measured. At the end of the month, the presence of flowering was then recorded for each tree. From this, the relationship between tree height and flowering could also be tested. Unfortunately this same study could not be carried out for pruned trees, as the availability of elephant damaged trees was too low.

4.2.2 Statistical analyses

For tree height and stem diameter, as well as for each measure of reproduction (e.g. pods/50 cm and pod mass), differences between utilised and non-utilised trees were determined using either Student's t-tests for normally distributed data, or Mann-Whitney U tests for data not normally distributed. The difference between tree height of flowering and non-flowering trees was also tested using a Mann-Whitney U test (Zar 1999).

4.3 Results

Defoliated and non-defoliated trees on which pod production was measured did not differ in height or largest stem diameter (Mann-Whitney U tests: Z = -1.27, *P* = 0.203 and Z = -0.231, *P* = 0.817 respectively). Similarly, the height and largest stem diameter of elephant utilised and non-utilised trees did not differ significantly (*t* = 1.93, df = 9, *P* = 0.086; *t* = 0.413, df = 9, *P* = 0.689 respectively). Trees were therefore comparable, as reproduction would not have been influenced by tree size.

As observed by Smit and Rethman (1998), some trees produced very few pods even though they had flowered. This was not related to previous utilization however, as four defoliated and four non-defoliated trees produced <10% pods, as did one pruned and two non-pruned trees. For trees with >10% pods, the mean (± SE) number of leaves/50 cm of branch was significantly less on defoliated than non-defoliated trees (28.5 ± 2.11 and 47.5 ± 2.11 respectively), while the mean number of pods did not differ (Table 4.1). The leaf:pod ratio was consequently higher in non-defoliated trees (6.64 ± 1.94), but not significantly different to that of defoliated trees (3.09 ± 0.41). There was also no difference in the leaf:pod ratio in elephant pruned trees compared to non-pruned trees, as the number of leaves or pods/50 cm of branch were no different (Table 4.1).

The leaf:pod ratio per branch was often highly variable within a tree, especially for defoliated trees. One tree, for example, had a branch with 44 pods and no leaves, and another with 4 pods and 68 leaves. Mean values per tree would hide this branch-level variability, which is perhaps a better indicator of the relationship between regrowth and reproduction. Therefore, leaf and pod production was also considered at the branch level, while bearing in mind that no real conclusions can be made from these analyses, as branches from the same tree were not independent of each other. When all branches were considered individually, the leaf:pod ratio was significantly lower in both defoliated and pruned trees compared to unutilised trees. The response in leaf and pod number was, however, different for the two utilization types. Defoliated trees had a significant reduction in leaf number/50 cm of branch and no increase in pod number, while pruned

Table 4.1 Pod and leaf production in mopane trees previously defoliated by mopane caterpillars or pruned by elephants, compared to unutilised trees (mean ± SE values).

Utilization type	Control trees		Utilised trees	*t* or $Z^{\#}$ value	*P* - value
Leaves/50cm					
Caterpillar	47.5 ± 3.43	>	28.5 ± 2.11	4.72	< 0.010
Elephant	53.6 ± 8.84	=	44.5 ± 2.61	$0.365^{\#}$	0.715
Pods/50cm					
Caterpillar	13.1 ± 2.79	=	18.2 ± 2.81	- 1.28	0.223
Elephant	16.0 ± 2.29	=	23.6 ± 3.69	- 1.80	0.106
Leaves/pods					
Caterpillar	6.64 ± 1.94	=	3.09 ± 0.41	1.99	0.070
Elephant	6.50 ± 1.95	=	3.34 ± 1.03	1.35	0.211
Pod mass (g)					
Caterpillar	0.328 ± 0.017	=	0.281 ± 0.020	1.77	0.095
Elephant	0.305 ± 0.025	=	0.336 ± 0.039	0.67	0.514
Seed mass (g)					
Caterpillar	0.077 ± 0.014	=	0.074 ± 0.013	0.148	0.884
Elephant	0.079 ± 0.012	=	0.089 ± 0.016	$0.575^{\#}$	0.565

The symbol $^{\#}$ indicates a *Z* value from a Mann-Whitney U test (for non-normally distributed data), as apposed to a *t*-value from a t-test (for normally distributed data).

trees produced the same number of leaves but significantly more pods than non-pruned trees (Fig. 4.1).

Mean values of pod and seed mass were both slightly lower after defoliation and greater after pruning, but masses were not significantly different (Table 4.1). On the tree level, reproductive investment (measured as pod density per branch and pod or seed mass) was therefore unaffected by either defoliation in the same season or recent pruning by elephants.

Pods on trees previously utilised by elephants tended to be less parasitised than pods on unutilised trees (17.1 % and 38.6 % respectively), and this difference was nearly significant (Mann-Whitney U test, $Z = -1.85$, $P = 0.064$). A similar trend was found for the mean values from defoliated (7.78 %) and non-defoliated trees (18.9 %), yet these values were not significantly different due to the high degree of variability between trees (ranging from 0-60% in control trees; Mann-Whitney U test, $Z = 1.23$, $P = 0.216$).

For the 80 defoliated and non-defoliated trees identified, the proportion of defoliated trees that flowered was nearly equal to that of non-defoliated trees (24 % versus 21 % respectively; total N of flowering trees = 18). Defoliation therefore had no influence on a trees likelihood of flowering in the same season. Flowering was, however, related to tree height, as flowering trees were significantly taller than non-flowering trees ($\bar{x} = 4.49$ m ± SE 0.30, $\bar{x} = 3.29$ m ± SE 0.17 respectively; Mann-Whitney U test: $Z = -3.24$, $P < 0.01$).

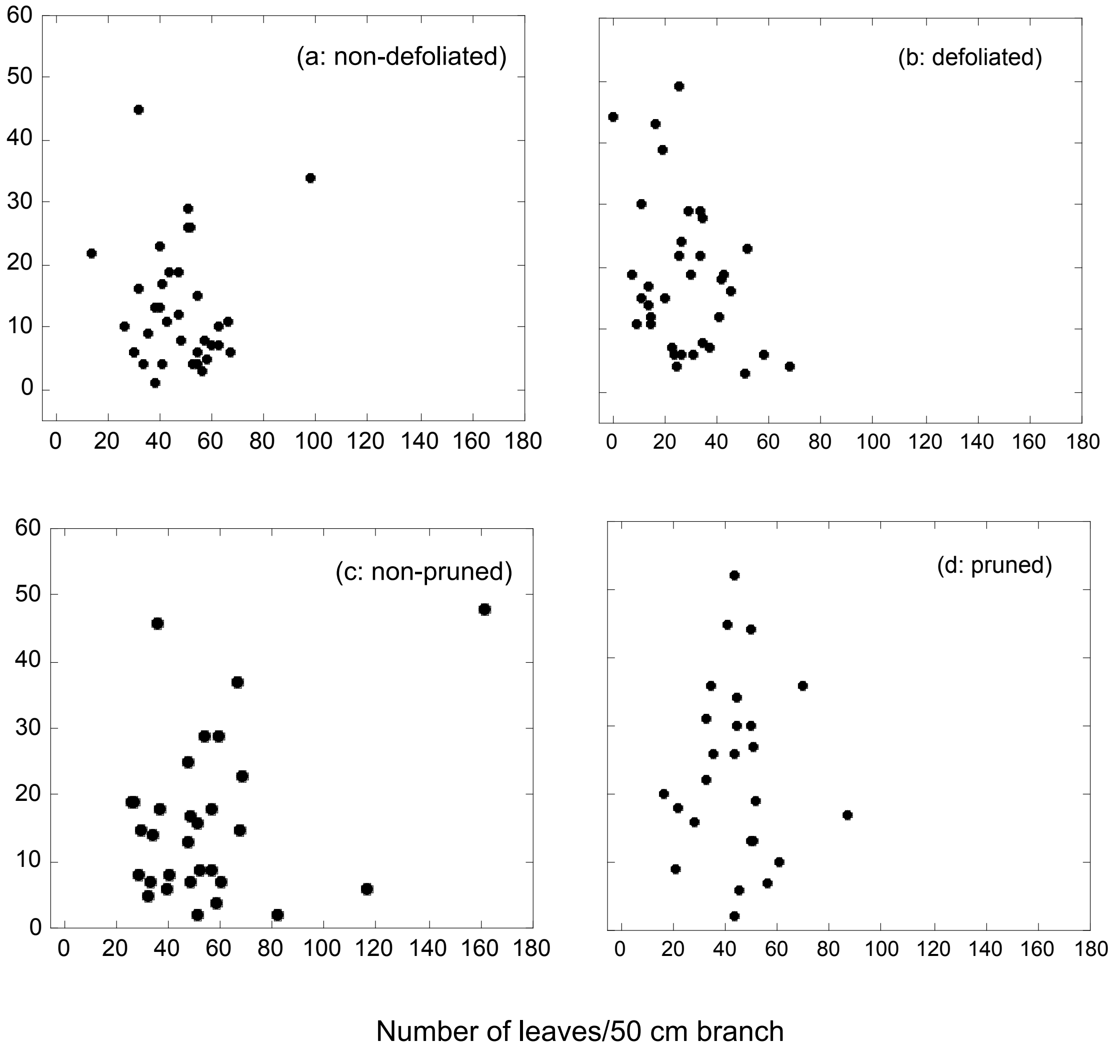

Figure 4.1 The number of leaves versus pods per 50 cm length of branch on (a) non-defoliated, (b) mopane caterpillar defoliated, (c) non-pruned and (d) elephant pruned mopane trees. Data from all branches are shown here (5 branches were sampled per tree).

4.4 Discussion

From this study, defoliation by mopane caterpillars and pruning by elephants appear to have no effect on the reproductive investment of mopane trees, as mean pod density and pod mass on utilised trees was no different to unutilised trees. Defoliation also had no influence on a plants' likelihood of flowering that season, with flowering being determined more by tree height. Unlike pod production, however, the mean leaf density was significantly reduced in the regrowth of defoliated trees.

The lack of negative impact on reproduction after herbivory found here is in accordance with results from studies on other species, such as *Ilex aquifolium* (Obeso 1998) and *Styrax obassia* (Tamura & Hiura 1998). As suggested by Marquis (1992), a possible reason for this apparent tolerance to browsing is that resources required for reproduction are not provided by current photosynthetic material, but rather by stored resources from branches or the main trunk. In mopane, flowering generally takes place shortly after the first defoliation event, before regrowth begins. Additional stored resources would therefore not yet have been utilised for regrowth, and the amount available for reproduction would remain unaffected. This would also explain why defoliation had no influence on a plants likelihood of flowering that season, nor any affect on whether a flowering plant produced pods or not.

Unlike defoliated trees, regrowth on elephant utilised trees had taken place before the onset of flowering, yet pod density and mass were still not negatively affected. Instead, pod number tended to increase on individual branches and the mean pod weight was slightly greater than on non-pruned trees. The possible negative effect that the early timing of elephant utilization could have had on pod production therefore seems to have been over-ridden by the increase in resource availability per branch (due to the increase in the root/shoot ratio caused by browsing; Danell *et al*. 1994). Similarly, the decrease in leaf density in the regrowth of defoliated reproductive trees can also be explained according to changes in stored resource availability. Here the expending of stored resources on reproduction prior to the onset of regrowth would have resulted in fewer resources available for leaf growth. Such a cost of reproduction has also been reported in the absence of herbivory, as Miyazaki *et al.* (2002) reported a reduction in leaf size on reproductive branches of *Styrax obassia* for example, and Bauce and Carisey (1996) found flowering Balsam fir (*Abies balsamea*) trees to produce half the current-year foliage of non-flowering trees. It should be noted, however, that from this study it cannot be concluded that the decrease in leaf number was in fact due to resource allocation towards reproduction, as a comparison with leaf number on defoliated, non-flowering trees could not be made.

The ability of mopane to invest in reproduction, even in the face of a stress such as herbivory, is possibly due to the species' pre-required store of resources necessary for reproduction to take place. For example, trees only begin flowering once large enough that the cost relative to the amount of stored resources is sufficiently low and hence affordable. Additionally, reproduction in individual mopane trees is sporadic, sometimes only re-occurring after four years. This too is presumably to allow the plant time to sufficiently build up its' store of resources between each reproductive event. Plant fitness would therefore largely be determined by the rate of resource accumulation within a plant, as this would affect the frequency of reproductive years. Consequently, factors influencing the rate of resource accumulation, such as soil type and inter-plant competition, could be important determinants of plant fitness (Maschinski & Whitham 1989), together with a plants' physical characteristics, such as leaf size and number. Defoliation and pruning, which cause significant changes in leaf size and shoot length (depending on the timing and frequency of damage; Chapter 3), would therefore also have a more long-term impact on mopane tree fitness. Ultimately, a long-term study is therefore needed to determine the true impact of herbivory on mopane reproduction (Doak 1992).

Interestingly, when all branches were considered individually, the relationship between leaf and pod number became far more apparent, with both types of utilization resulting in a decreased leaf:pod ratio. According to Honkanen and Haukioja (1994), partial defoliations create asymmetric within-canopy competition over resources, with defoliated branches being less competitive and thereby displaying retarded growth. Additionally, the movement of resources between branches is restricted in woody plants, meaning that a defoliated branch would suffer more resource depletion after flower production than non-defoliated branches (Marquis 1992; Alados *et al.* 1997). Leaf and pod production within a single tree canopy is therefore often variable, depending on damage locality. In this study, trees were not completely defoliated (ranging from 50 to 95%) and non-defoliated sections were not marked as such. Sampled branches could therefore have been from both defoliated and non-defoliated areas, or from damaged and undamaged areas in pruned trees. Thus, it is not surprising that the leaf - pod relationship was more significant at the branch level, as branch-specific responses would then have been detected. I therefore recommend that future studies take note of the previous utilisation of specific branches within a tree to better detect the impact of herbivory, and to confirm the apparent branch-specific response in reproduction observed here.

It should be noted that pod production in this study was measured as the density of pods per branch, and was not a whole tree count. Therefore, even though mopane appears to display overcompensation in reproduction at the branch level after pruning, total pod production may still have been unaffected (or negatively affected) by a reduction in tree size.

The lack of significant change in seed mass after pruning, but the increase in seed number (at the branch level), is similar to results from studies on *Gentianella campestris* (Lennartsson *et al*. 1998) and *Ipomopsis aggregata* (Paige 1992). This suggests that the trade-off in resource allocation between seed size and number is not affected by changes in resource availability caused by herbivory (Geritz *et al*. 1999; Kiviniemi 2001; Leishman 2001). There was, however, a slight increase and decrease in pod weight after pruning and defoliation respectively, and these differences may also become significant if considered at the branch level (i.e. pods collected specifically from utilised and unutilised branches). If this does occur, and if seed size and seedling recruitment in mopane are positively related, defoliation and pruning could then have a negative and positive influence the reproductive success of mopane trees respectively.

Apart from seed size, an additional factor affecting seed viability is pre-dispersal damage caused by seed predatory insects (Igarashi & Kamata 1997). For example, infestation of *Acacia erioloba* pods by bruchid beetles was found to be the primary factor preventing seed germination under conditions of adequate water (Barnes 2001). Similarly, insect infested acorns from holm-oak trees (*Quercus ilex*) had a 15 percent decrease in viability (Leiva & Fernández-Alés 2005). While the mean percentage of damaged pods tended to be lower on browsed mopane trees, the reliability of this trend is questionable as there was a high degree of between-tree variability within each treatment. Such variability has also been reported for other species (Barnes 2001; Leiva & Fernández-Alés 2005), but the cause is not well understood. In this study, both the sample size of pods per tree and trees per treatment were relatively small, which could be responsible for the high degree of variability. Further work with larger sample sizes is therefore recommended to confirm this interesting trend, as this knowledge could help our understanding of the indirect impact of herbivory on plant fitness.

In conclusion, mopane trees appear tolerant of defoliation by mopane caterpillars and pruning by elephant (if no change in canopy size) in the short term, as the ability to invest in reproduction remained unaffected. Defoliation also had no effect on a trees' likelihood of flowering that season, which was instead determined by tree height. Furthermore, although not specifically tested in this study, production of pods and leaves appears to be dependant on damage at the branch level, indicating the lack of resource movement between branches and hence the importance of localised resource availability. Reproduction in mopane trees therefore seems to be determined by stored resources in the plant, and is not altered in response to herbivory. The only negative effect recorded here was the decrease in leaf density in regrowth after defoliation. This could potentially result in a delayed or long-term negative impact on reproduction, as the rate of resource accumulation and hence the frequency of reproduction could be affected. Similarly, elephant pruning could also have a long-term negative impact on plant fitness if trees are prevented from reaching a reproductive size.

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CHAPTER 5

INTRASPECIFIC HOST PREFERENCES OF MOPANE MOTHS (*IMBRASIA BELINA*) IN MOPANE *(COLOPHOSPERMUM MOPANE*) WOODLAND

5.1 Introduction

Fundamental to the life cycle of most phytophagous insects is the finding of a suitable plant for oviposition, especially for species with relatively immobile larvae. Frequently, it is assumed that females select host plants that provide qualitatively and quantitatively the best food for their larvae, as natural selection should favour a positive relationship between adult oviposition and offspring performance. However, while numerous studies do support this expectation (Rausher 1981; Denno *et al.* 1990; Dini & Owen-Smith 1995; Lower *et al*. 2003), a review of the literature has found that nearly half of studies found no such correlation (Mayhew 1997). Instead, oviposition may be influenced by other factors such as: predator avoidance for offspring (Denno *et al*. 1990; Mappes & Kaitala 1995; Björkman *et al.*1997; Mira & Bernays 2002; Shiojiri *et al*. 2002; Nomikou *et al*. 2003); adult survival and performance (Mayhew 2001; Scheirs 2002); host distribution (Ballabeni *et al*. 2001), host patch size or density; and plant architecture (Marquis *et al*. 2002). Preferences may therefore be determined by trade-offs among multiple factors (Bernays & Graham 1988).

Until now, the focus of most host-preference studies has been to try and gain a better understanding of a species’ host range, and have therefore looked at interspecific preferences (Wehling & Thompson 1997; Carrière 1998). In a habitat where the preferred host species dominates, however, interspecific host choices are largely irrelevant and instead, intraspecific preference is expected to be of greater importance. Surprisingly, however, very little work has looked into this. In carrying out such a study, intraspecific variables (e.g. leaf size and shoot length) would require investigation so that preference may be detected at a finer scale of resolution, as variation within a trait is likely to be less within one species than across multiple species.

In mopane woodland, *Colophospermum mopane* trees (commonly known as 'mopane', see Chapter 2 for details) tend to dominate and generally comprise 90% of the total biomass (Guy 1981). Mopane is the main host species of the larvae of the mopane moth (*Imbrasia belina*, see Chapter 2 for details) and, where dominant, it may be fed on exclusively (Pinhey 1972; personal observation). Within mopane woodlands, outbreaks of mopane caterpillars are also common at certain times of the year (November/December and February/March), resulting in large stands of trees being completely defoliated (Ditlhogo 1996).

Although species diversity does not vary much within mopane woodland, a high degree of variability does exist in other ways, such as in the growth form and density of trees. While mopane usually ranges from about 10 m in height, with large areas of low scrub of 1-2 m, stands of trees up to 20 m high exist and are termed "cathedral mopane" (Van Wyk 1993). Associated with these height differences is a difference in plant architecture, as shrubs are usually multi-stemmed while taller trees tend to be single-stemmed (Fraser *et al*. 1987). The principle cause of these variations in tree height has been identified to be variations in the soil, particularly depth and pH (O'Connor 1992). Variations in foliar chemical composition may therefore also be expected between habitat types. Furthermore, densities of mature mopane woodland vary greatly, ranging from a few trees per hectare in arid north-western Namibia (Viljoen 1989) to 481 trees ha^{-1} in south-eastern Zimbabwe (Kelly & Walker 1976) and 2,740 trees ha^{-1} in northern South Africa (this study). This high degree of variation within mopane woodlands thus results in very different habitat types which may, in turn, be expected to influence host preference by mopane moths at the habitat level.

Host preference at the tree level may also be important for mopane moths, as the mobility of larvae affects the way in which adults perceive the vegetation. For species that move readily between plants while feeding, the vegetation may be perceived as a single population with average or aggregate attributes, for example, while parasitic species (confined to one or a few trees) may see the vegetation as a collection of plants with individual traits (Edelstein-Keshet & Rausher 1989). The relative immobility of mopane caterpillars may therefore increase the moth's sensitivity of preference to the tree level. Additionally, selective oviposition may have evolved in mopane moths to reduce intraspecific competition for resources during outbreak sessions, as females could adjust the number of eggs laid according to the host quality and density of conspecifics (Tammaru *et al*. 1995). Therefore, unlike the majority of phytophagous species, where competition for food is considered unimportant (Jaenike & Holt 1991), outbreak species may have evolved the ability to select hosts based on resource quantity, rather than quality. However, a low degree of selectivity may also be expected for an outbreak species, as according to Price (1994), eruptive population dynamics of phytophagous insects could be due to a lack of selectivity by ovipositing females, as there is no within-generation feedback between deteriorating food resources and natality.

While looking at the effects of host preference (e.g. larval performance) is not in the scope of this project, investigating the degree of selectivity displayed by mopane moths and identifying what mopane tree characteristics determine this selectivity will help improve our understanding of the ecology of insect-plant relations, specifically for species achieving outbreak dynamics within monospecific tree stands. Additionally, understanding what factors influence the distribution and abundance of mopane caterpillars has socioecological value, as the edible caterpillar forms an important food source to rural communities and has become an important trading commodity (Munthali & Mughogho 1992).

The aim of this study was therefore to investigate intraspecific host preference by mopane moths within mopane woodland, by addressing the following three main questions: (1) Do ovipositing females display host preference at (a) the habitat level and (b) the individual tree level? (2) If so, which tree characteristics determine this preference? (3) Does resource quantity (tree size) influence host preference for oviposition in this outbreak species?

5.2 Methods

5.2.1 Study site

The study was carried out in the Venetia-Limpopo Nature Reserve (i.e. Venetia, see Chapter 2 for details). While there is no “cathedral” mopane within Venetia, the mopane community may still be divided into three different habitat types, namely: short scrub, medium height woodland and tall riverine habitats, with mean heights of 1.1 m, 2.5 m, and 5.6 m respectively.

In late November to early December of 2002, just after the first batch of mopane moths had emerged and laid their eggs, five representative areas for each of the three habitat types (scrub, woodland and riverine) were identified and a transect set out within each (i.e. 15 transects). All transects were 5 m wide and either 100, 50 or 25 m in length, depending on tree density (longer transects were required in low density areas in order to include enough trees) and habitat patch size (some riverine areas were particularly small). To quantitatively describe each area, detailed information was recorded for the first 25 –

35 trees along each transect, depending on tree density. For one riverine transect, only 19 trees were sampled due to the very low tree density.

5.2.2 Habitat description

The following information was recorded for each tree on each transect: live tree height (m), basal stem diameter (cm), number of live stems, canopy width at the widest point (m) and canopy height (m). A rough estimate of canopy volume was calculated for each tree as the volume of a cylinder with canopy height and width dimensions. For each transect, mean tree height, mean stem diameter, mean stems per tree and total canopy volume/ha were then determined. Tree density/ha was also calculated, by counting the total number of trees within the transect area. Additionally, the mean nutritional value of foliage (protein, tannin and total polyphenolic content) in a transect area was determined by calculating the mean value from individual trees sampled in section 5.2.4.

5.2.3 Host preference – habitat level

Within each transect, all trees with mopane caterpillar egg masses from the current season were identified as host trees and tagged. Egg mass abundance per tree was recorded on a scale of 0-3, where 0 = 0 egg masses, 1 = 1 egg mass, 2 = 2 egg masses and 3 = 3 or more egg masses. The percentage of host trees was calculated for each transect, together with the total number of egg masses/ha. Estimates of egg mass densities were likely to be accurate in scrub and woodland areas, as trees were small enough to search thoroughly and the exact number was deducible from most egg mass scores. In woodlands, for example, only 2 out of 63 trees had a score of 3. However, in riverine areas, large tree size and the high number of ‘3’ scores (which could mean more than three egg masses) prevented accurate density calculations. The degree of error also presumably increased with increasing tree size.

The term ‘preference’ is defined by deviation from random behaviour, where a resource is utilised significantly more than expected in relation to its availability (Singer 1986). To specifically test the prediction that mopane moths would prefer a certain habitat type, the relationship between egg mass number/ha and available canopy volume/ha was investigated. Yet, due to the high probability of underestimated egg counts in riverine trees, this habitat could not be included in the test. Only woodland and scrub areas were therefore compared in this way. However, when looking at the habitat characteristics that may determine habitat selection, the ‘percentage host trees’ and the number of egg masses/ha were compared to (1) tree height, (2) tree density, (3) canopy volume/ha and (4) leaf nutritional value of all three habitat types, to look for similar or opposite trends.

5.2.4 Host preference – tree level

For each host tree, the nearest conspecific neighbour without egg masses was identified and tagged. This enabled a comparison between trees used as oviposition sites by moths, compared to those that were also present in the area, but not used. Non-host trees were not necessarily within the transect area. Tree height, stem circumference and canopy width and height were recorded for all host and non-host trees.

For more detailed comparisons between host and non-host trees, additional data were collected from five tree pairs (host and non-host) per transect. Leaf size was measured for 15 mature, undamaged leaves, collected at random from all sides of the canopy. The length of the right leaflet was measured in millimetres from the growth point to the leaf apex. The length of the current year’s shoot growth (which was easily identifiable from the apparent growth point) was measured for 15 shoots per tree to the nearest centimetre. To estimate shoot biomass, 15 shoots were clipped and subsequently weighed after oven drying (at 50ºC) to a constant mass. The number of shoots on the entire tree was then estimated by counting the exact number within a volume of canopy, and multiplying this by the estimated proportion of the canopy the sample volume represented. Total shoot mass was then calculated accordingly. Shoot density was measured as the number of shoots on the terminal 50 cm of a branch, for five branches per tree. Additionally, leaves were collected for chemical analysis, where the protein, tannin and total polyphenolic content was determined (see method details in Chapter 3).

To test the hypothesis that host selection by this outbreak moth species would be affected by resource quantity at the tree level, trees within riverine and woodland transects were divided into four and three 'canopy volume' classes respectively. Riverine classes included: (1) 0-50, (2) 51-100, (3) 101-200 and (4) > 200 m^3, while woodland classes included: (1) 1-10, (2) 11-20 and (3) > 20 m^3. Only transects with 75% host trees were considered (i.e. three for each habitat), and trees from like-habitat transects were grouped together.

The total number of egg masses found within each canopy class was then determined and compared to the number expected. For woodlands, the expected number was first calculated according to the number of trees within each class and secondly, according to the total canopy volume available within each class. Utilization of different size trees in relation to the number available could therefore be determined, together with preference based on actual resource availability. Once again, however, this was only possible for woodland areas. For riverine areas, the expected number of egg masses was only calculated according to tree number (due to inaccurate egg density estimations).

5.2.5 Statistical analyses

Variation across habitat types in each variable (shoot length, leaf length etc.) was analysed using a single factor ANOVA. When significant variation was found, post hoc Tukey tests were carried out to determine between which habitats significant differences occured. Data were Log transformed when not normally distributed.

Most data describing individual trees were not normally distributed. For each descriptive variable, Wilcoxon matched-pairs tests were therefore used to test for differences between host and non-host trees.

Chi-squared goodness-of-fit analyses were used to determine whether certain canopy volume classes were used disproportionately to their availability, both in terms of tree number and total canopy volume (Zar 1999). Where significant variation was found, Bonferroni confidence intervals were used to determine which classes were preferred or avoided (Miller 1966; Neu *et al*. 1974; Byers & Steinhorst 1984).

5.3 Results

5.3.1 Habitat description

The three mopane habitat types differed significantly in mean tree height, canopy volume/ha and the number of live stems/tree. Riverine areas consisted of significantly taller trees with fewer stems and a greater canopy volume/ha than woodland or scrub areas, while woodland areas consisted of taller trees with a greater canopy volume/ha than scrub areas (Table 5.1). Tree density did not appear to differ between habitat types, however, when one riverine transect with an unusually high density (2720 trees/ha compared to a mean of 850 trees/ha) was excluded from the analysis, tree density in riverine areas was significantly less than in woodland and scrub areas. Foliar nutritional

Table 5.1 The mean ± SE values of variables describing the three mopane habitat types found in the Venetia-Limpopo Nature Reserve, namely: riverine, woodland and scrub mopane.

Variable	Riverine	Woodland	Scrub
Habitat description			
Tree height (m)	6.11 ± 0.43	2.50 ± 0.23	1.07 ± 0.06
Canopy volume/ha (m^3)	1898 ± 433	280 ± 60	56 ± 7
Alive stems/tree	2.37 ± 0.31	4.69 ± 0.47	4.06 ± 0.27
Trees/ha	1224 ± 389	2060 ± 340	2092 ± 111
Tannin:protein ratio	0.49 ± 0.05	0.74 ± 0.07	0.55 ± 0.09
Total polyphenols (mg/g)	56.0 ± 2.71	73.2 ± 4.63	60.5 ± 7.40
Utilization			
% host trees	38.9 ± 15.9	10.6 ± 5.23	0.44 ± 0.27
Egg masses/ha	632 ± 306	224 ± 87.7	8.00 ± 4.90

value did not differ between habitat types, as there were no differences in the tannin:protein ratio or total polyphenolic content.

5.3.2 Host preference – habitat level

Riverine mopane had, on average, the highest percentage of host trees (38.9%), followed by woodland (10.6%) and lastly scrub mopane (0.5%), where only two trees with egg masses were found (Table 5.1). These differences were not quite significant ($P = 0.078$), yet this is most likely due to the high degree of variability within riverine (0-79 %) and woodland areas (0-29%). Both these habitats had two transects containing less than 5% host trees, indicating that *I. belina* may simply not occur in some areas. Excluding these unutilised areas, the percentage of host trees rose to 17% in the woodland and 64% in the riverine areas. Similarly, the number of egg masses/ha rose from 224 and 632 to 366 and 1040 in woodland and riverine areas respectively. The far greater number of egg masses/ha in riverine areas (which is likely to be underestimated) indicates that the greater percentage of host trees there is not simply due to an equal number of eggs being distributed among fewer trees. Looking specifically at habitat preference, woodlands were preferred over scrub areas ($\chi^2 = 58.5$, df = 1, $P < 0.01$; riverine habitat not included in analysis). Mopane moths do therefore appear to display host preference at the habitat scale**.**

The pattern of host tree abundance and egg mass density decreasing from riverine to woodland to scrub mopane matches that of tree height and canopy volume/ha, but none of the other descriptive variables (Table 5.2). The increase in the percentage of host trees with increasing mean tree height within a transect is clearly shown in Figure 5.1. Since both tree height and canopy volume/ha are indirect measures of foliage biomass, it

Table 5.2 Results from ANOVAS and Tukey tests for variables describing differences between riverine, woodland and scrub mopane habitat types.

Variable	Habitat comparison	*P*- value
Tree height	riverine > woodland > scrub	< 0.001
Canopy volume/ha	riverine > woodland > scrub	< 0.001
Tree density#	woodland > scrub = riverine	0.006
Stems/tree	woodland = scrub > riverine	0.002
Tannin:protein ratio	woodland = scrub = riverine	0.072
Total polyphenolics	woodland = scrub = riverine	0.096
% host tress	(riverine > woodland > scrub) §	0.078

Excluding one riverine transect with an unusually high tree density.

§ Relationship is shown, even though not significant (explanation given in text)

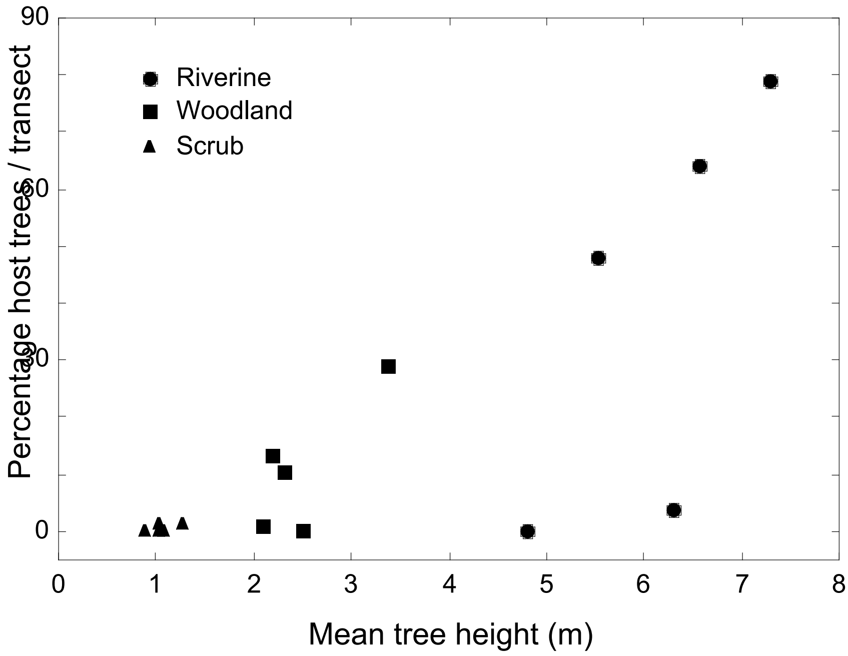

Figure 5.1 The percentage of host trees per transect versus the mean tree height of the corresponding transect.

appears that resources quantity, rather than quality, is the determinant of habitat preference by mopane moths.

5.3.3 Host selection – tree level

There were no differences in shoot length or leaf nutritional value between host and non-host trees. Individual host trees were, however, significantly taller with a larger shoot biomass than their neighbouring non-host tree in both riverine and woodland habitats (Table 5.3). In riverine areas, host trees also had significantly heavier shoots and larger leaves, yet this is most likely due to the corresponding greater leaf size and shoot length associated with larger trees (relationships with tree height: $F_{1,55} = 39.1$, $P < 0.01$, $R^2_{adj} = 0.40$ and $F_{1,54} = 27.6$, $P < 0.01$, $R^2_{adj} = 0.33$ for leaf size and shoot length respectively). Tree size therefore also seems to be the primary factor influencing oviposition at the tree level.

Confirming these results, the number of egg masses per tree was found to be significantly related to tree height ($F_{1,4} = 124$, $P < 0.01$, $R^2 = 0.961$; Fig. 5.2). Considering the habitat types separately, in riverine areas, which are dominated by tall trees, trees as tall as 4-5 m were hardly utilized. Most host trees were > 6 m in height, on which egg mass abundance only increased slightly with increasing tree height (Fig. 5.3a). In woodland areas, however, where the mean tree height was only around 2.5 m, the increase in egg mass number with increasing tree height was far more apparent (Fig. 5.3b). Here, an egg mass score of 3 was only found on the tallest trees, with a mean height of 4.2 m. Egg abundance per tree is therefore related to relative tree height in an area.

5.3.4 Host preference – canopy volume

Based on the number of trees available in each size class, trees from the smallest canopy volume class (0-50 m^3) were avoided, while those from the largest canopy class

Table 5.3 Results from Wilcoxon matched-pairs tests, comparing characteristics of host and non-host mopane trees in woodland and riverine habitats.

Variable	Habitat type	Host vs. non-host trees	*P*- value
Tree height	riverine	host > non-host	< 0.01 **
	woodland	host > non-host	< 0.05 *
Total shoot biomass	riverine	host > non-host	< 0.01 **
	woodland	host > non-host	< 0.05 *
Shoot weight	riverine	host > non-host	< 0.05 *
	woodland	host = non-host	1.00
Shoot length	riverine	host = non-host	0.14
	woodland	host = non-host	0.16
Leaf length	riverine	host > non-host	< 0.01 **
	woodland	host = non-host	0.78
Tannin: protein ratio	riverine	host = non-host	0.57
	woodland	host = non-host	0.53
Total polyphenolics	riverine	host = non-host	0.36
	woodland	host = non-host	0.10

* Indicates significance at the 5% (*) or 1% (**) level

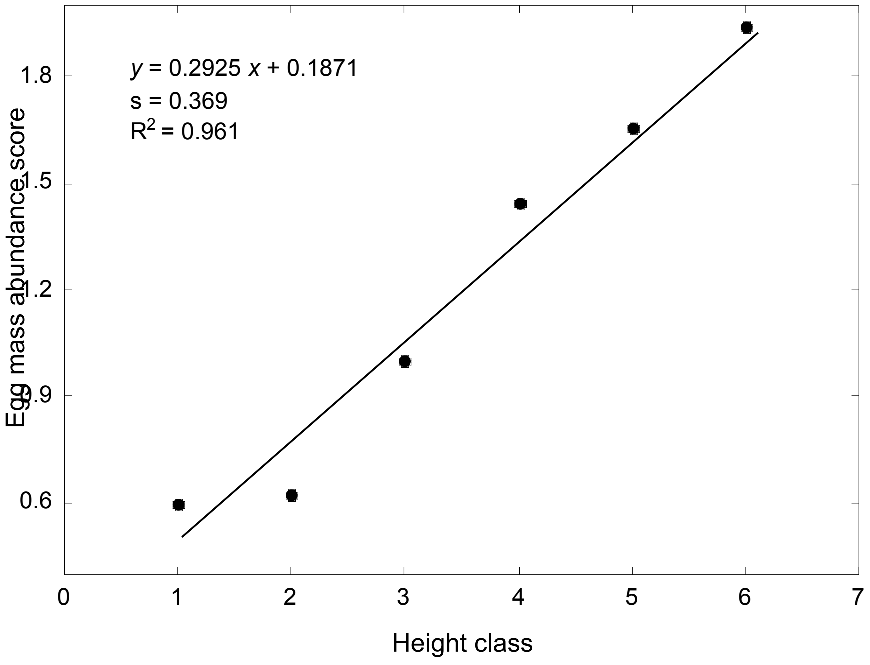

Figure 5.2 The relationship between the number of egg masses per tree (score 0-3) and tree height. Heights are grouped into the following classes: 1 = 0 - 1.5 m, 2 = 1.6 - 3 m, 3 = 3.1 - 4.5 m, 4 = 4.6 - 6 m, 5 = 6.1 - 7.5 m, 7 = > 7.5 m.

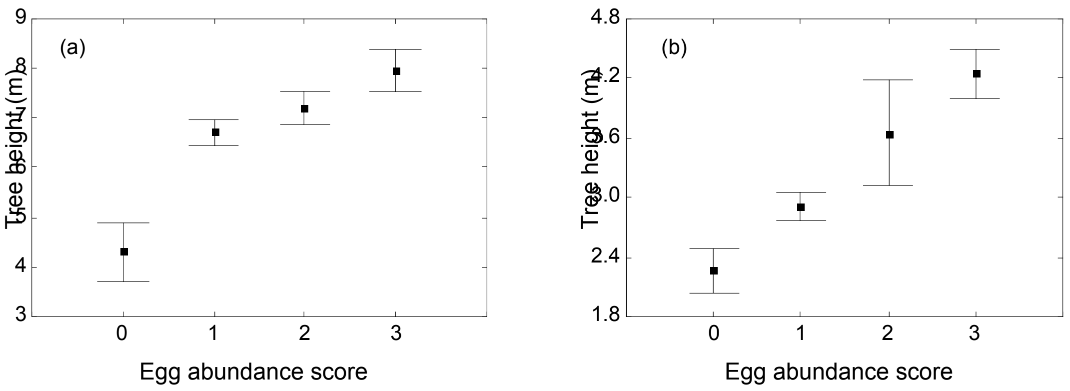

Figure 5.3 The mean (± SE) height of trees for each egg abundance score in mopane (a) riverine and (b) woodland habitats.

Table 5.4 The preference for different size mopane tree canopies by ovipositing mopane moths, firstly based on the number of trees within a canopy volume class and secondly, on the total canopy volume within a class.

Habitat	Canopy volume (m^3)	Observed egg masses	Expected egg masses - trees/class (preference)[#]	Expected egg masses - total volume/class (preference)[#]
Riverine	0-50	17	34 (-)	Not applicable
	51-100	20	18 (0)	
	101-200	27	21 (0)	
	>200	20	11 (+)	
Woodland	0-10	5	16 (-)	6 (0)
	11-20	8	6 (+)	7 (0)
	>20	14	5 (+)	14 (0)

Symbols indicate classes used significantly more (+), less than (-), or equal to their availability (0), determined by Bonferroni confidence intervals.

(> 200 m^3) were preferred in riverine areas (Table 5.4). Similarly, in woodland areas, smallest canopy trees (0-10 m^3) were avoided and both the larger canopy classes were preferred (11-20 m^3 and > 20 m^3). However, when considering the actual resource availability in each class instead of tree number, no canopy size class was preferred or avoided. Instead, each was utilised as expected in relation to its availability, as can be seen in Table 5.4.

5.4 Discussion

Ovipositing mopane moths were found to display host selectivity at the habitat scale, as egg densities were highest in riverine areas and the scrub habitat was avoided compared to the woodland habitat. Tree size was determined as the primary factor influencing habitat preference, and individual host trees were also significantly taller than non-host trees. Additionally, the number of egg masses per tree increased with increasing tree size. Tree size therefore appears to be an important determining factor of oviposition by mopane moths. However, preference for larger canopied trees at the tree level was not found, as egg mass density was as expected according to available canopy volume within each canopy size class. The greater number of egg masses on taller trees within an area was therefore probably due to an increased chance of moths intercepting large trees, rather than actual preference.

It should be noted, however, that this direct relationship between egg mass density and available canopy volume may also be interpreted as a display of high selectivity. Studies have shown that oviposition behaviour by phytophagous insects is often modified by the presence of conspecific broods (eggs and larva), with females typically avoiding depositing eggs on previously exploited host resources (Nufio & Papaj 2001). Females may be able to distinguish between occupied and unoccupied hosts by various stimuli, such as the visual cue of an egg mass or a signal received through marking pheromones (Schoonhoven 1990). Such behaviour is thought to have evolved to reduce the competition for resources between their offspring (Prokopy 1981). For an outbreak species such as *Imbrasia belina*, that experiences resource limitation, moths could therefore have adopted this behaviour. Additionally, density-dependence can increase the preference for low ranking hosts if natural selection favours a broad oviposition strategy, where low-quality plants are accepted due to a reduced probability of offspring survival on densely populated higher quality plants (Mayhew 1997). Both these factors could therefore explain the apparent lack of preference for larger canopies on the tree level, and this would mean that mopane moths are not only selecting trees according to their initial resource availability (based on tree size), but also the relative availability through conspecific density.

According to Jaenike (1990), host plant abundance influences search time and specialisation. The high density of mopane trees would therefore allow for an unusually high degree of selectivity by mopane moths, despite their short adult life span, as search time for the correct species would be very low, thereby allowing more time for intraspecific specialisation. To test this, however, changes in oviposition behaviour with increasing egg mass densities should be investigated.

Apart from tree size, leaf size of host trees was also significantly larger than on non-host trees. While this would have had a positive affect on resource availability, the degree of influence on oviposition behaviour is questionable however, as such changes in total biomass per tree would be far more subtle and difficult to detect compared to changes in tree size. Additionally, preference based on tree size was not even found at the tree level, making preference based on a more detailed tree characteristic such as leaf size unlikely. A trait such as leaf size is, instead, expected to be more important for small immobile species, where individuals feed on only one or a few leaves. *Pemphigus* aphids, for example, were found to prefer large leaves on which the weight of stem mothers and their progeny was greater (Karban & Agrawal 2002). Mopane caterpillars, however, are relatively mobile in comparison and feed on a large number of leaves during their development. Additionally, the high degree of variability in leaf size on a single tree (Wiggins 1997; Chapter 7) would reduce the relevance of this trait.

With regards to leaf nutritional value and host preference, the lack of difference between leaf chemistry of individual host and non-host trees is not surprising, as very little variation was found between trees within the same habitat type. Foliar quality did, however, tend to differ between habitat types (although not significantly), yet habitat preference by mopane moths did not correspond to these differences. Woodlands, which had an intermediate level of preference, had a poorer foliar nutritional value than the unutilised scrub mopane. Assuming the measures of leaf chemistry measured here (tannin:protein ratio and total polyphenolics) are relevant to mopane caterpillar growth, it appears that host choice by mopane moths is therefore not determined by resource quality, but rather quantity.

An explanation for the apparent lack of importance of foliar quality could be that caterpillars have evolved various traits allowing them to handle the foliar chemical composition (Karban & Agrawal 2002). One such trait, for example, is that larvae may compensate for suboptimal foods by increasing their ingestion rate or duration of development (Schroeder 1986). Secondly, larvae could have various physiological and morphological traits enabling them to exploit their host plant, such as the production of enzymes (in the gut or saliva) that reduce the detrimental effects of potentially damaging plant compounds (Bernays & Chapman 2000). A third trait applicable to mopane caterpillars is their gregarious feeding behaviour when young, as this is known to enhance the ability of herbivores to exploit their host plants (Nahrung *et al*. 2001; Fordyce 2003). Denno and Benrey (1997), for example, found gregarious caterpillars to grow twice as fast in large groups (30 individuals) compared with smaller groups (1-10 individuals). Large groups are thought to either cause nutrient sinks or prevent induced defences in intact plants compared with smaller groups of caterpillars (Karban & Agrawal 2002). The adoption of such traits by mopane caterpillars is probable, as it would explain the species' ability to exploit a wide range of mopane trees and reach outbreak proportions.

While the key tree characteristic determining host preference by mopane moths has been identified as tree size in this study, it cannot be assumed that resource abundance alone is the only motivation behind this preference. The risk of predation, for example, identified as one of the most important influences on oviposition behaviour, could also play an important part (Lill *et al*. 2002). Mopane caterpillars are eaten by a wide range of species, including mammals (e.g. baboons, *Papio cynocephalus ursinus* and vervet monkeys, *Cercopithecus aethiops*), birds (34 species were documented by Styles 1995), and invertebrates (e.g. spiders, ants, and mantids; Styles & Skinner 1996). In a study by Kaitaniemi and Rouhomäki (2001), the disappearance of geometric moth larvae (*Epirrita autumnata*) in mountain birch (*Betula pubescens* spp. *czerepanovii*) was related to stem age (tree size). It was suggested it could be due to the smaller size and simple architecture of young stems, which may affect the probability of detection by predators. This too could then apply to mopane caterpillars on small mopane trees. Additionally, the greater resource availability per tree would prevent the need for larvae to migrate between trees, a time during which they are vulnerable to additional predators such as jackal (*Canis mesomelas*) and various ground birds.

Interestingly, in one of the few studies on intraspecific host preference, Björkman *et al*. (1997) also found a positive correlation between tree height and egg density for pine sawflies (*Neodiprion sertifer*) and their host species, Scots pine (*Pinus sylvestris*). They suggested that the preference for large trees could be due to microclimate. Within the range of mopane caterpillars, operative temperatures approaching the upper critical limit (43-48ºC) are common (Frears *et al*. 1997) and populations are known to die from desiccation (personal observation). Large canopied tress may therefore be preferred as they provide a larger amount of shade.

In conclusion, this study shows mopane moths to display a low degree of intraspecific host selectivity, as preference was only evident at the habitat scale. The apparent lack of host choice at the individual tree level; the lack of influence of resource quality on host choice; and the pattern of increasing egg mass density with increasing resource quantity (canopy volume) are all as one may expect for an outbreak species. Further work is, however, still needed to determine whether the direct relationship between tree size and egg density is driven by resource abundance, or other factors, such as predator avoidance. Similarly, the apparent lack of preference for large trees on the individual tree level is questionable, as it is possible that ovipositing females were actually avoiding conspecific egg masses to reduce intraspecific competition. Either way, identifying tree size as the primary determinant of oviposition not only helps in our understanding of the distribution and abundance of mopane moths within mopane woodland, but will also help in the future conservation of the species in localised areas, as management decisions can now be made accordingly to ensure suitable habitat is left intact.

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CHAPTER 6

ELEPHANTS AND MOPANE CATERPILLARS: INTERACTION THROUGH A SHARED RESOURCE

6.1 Introduction

An important determinant of an ecosystem’s structure and dynamics is the interaction between species for food (Brown & Davidson 1977). In the past, most studies looking at such interactions tended to focus on taxonomically related species, as they were the most obvious to compare due to their similarities. In reality, however, it is the functional similarity (i.e. species from the same guild) rather than the taxonomic similarity of species that is relevant. Additionally, while ecologists have long recognised the importance of direct interactions (such as competition and predation) in determining the distribution and abundance of organisms, the role of indirect interaction pathways has not been as well documented (Davidson *et al*. 1984). More recently, however, a growing number of examples have illustrated that indirect effects, such as habitat modification, may exceed the direct consequences of herbivory. For example, two taxonomically different herbivores, namely beavers (*Castor canadensis*) and leaf beetles (*Chrysomela confluens*), have been shown to interact indirectly through a common food source, cottonwood (*Populus* sp.; Martinsen *et al*. 1998).

In the mopane (*Colophospermum mopane*) woodlands of Southern Africa, the two main browsing species are a megaherbivore, the African elephant (*Loxodonta africana*), and an insect, the mopane caterpillar (*Imbrasia belina*). Despite its abundance, mopane is selected for by elephants and can form the dominant constituent of their diet in certain areas (Lewis 1991; Smallie & O’Connor 2000). Woodlands dominated by mopane are therefore susceptible to elephant induced damage through leaf stripping, branch/stem breaking and uprooting (Ben-Shahar 1993). In northern Botswana, for example, only 8.3% of mopane trees sampled had never been damaged by elephants (Ben-Shahar 1998). Elephants are therefore considered to be a 'keystone' species in these woodlands (Timberlake 1995), as they are a prominent agent of structural change to mopane (Caughley 1976). Similarly, mopane caterpillars can also have a significant impact on mopane trees. Well known for their episodic population outbreaks within mopane woodland, they often completely defoliate vast stands of trees (Ditlhogo 1996). Styles (1994), for example, found that in just six weeks, the mopane caterpillar population within his study area in northern South Africa consumed 9.4 times the amount of leaf material than the elephant population could have processed in a year. Clearly, these two species therefore not only both utilize mopane, but are also both capable of having a significant impact on the trees. It is thus to be expected that the feeding actions of one species should have a significant influence on the other.

A key difference between the two browsers is the type of damage they inflict upon mopane trees. While elephants utilise mostly branches and the main stems, thereby having a pruning effect on the trees (Smallie & O'Connor 2000), mopane caterpillars defoliate trees by removing just the leaf blades. While this differential use of plant parts might reduce direct competition between the two species, an indirect interaction may still result due to the different changes in plant morphology and chemistry known to occur after pruning and defoliation. In my study on mopane exposed to both simulated and natural elephant browsing and mopane caterpillar defoliation, plants were found to respond to pruning by producing longer shoots with larger leaves, while defoliation resulted in regrowth with shorter shoots and smaller leaves (see Chapter 3). These results were in accordance with numerous other studies on mammalian (Danell *et al*. 1994; Duncan *et al*. 1998; Bergström *et al*. 2000; Lehtilä *et al*. 2000) and insect herbivory (Gadd *et al*. 2001;

Anttonen *et al*. 2002; Piene *et al*. 2002). Unlike many woody plant species, however, the foliar nutritional value (measured as tannin/protein ratio and total polyphenolic content) was found not to change after pruning by elephant, yet increased after defoliation by mopane caterpillars.

Browsing behaviour of both mammals and insects is influenced by morphological and chemical plant characteristics (reviews by: Bryant *et al.* 1991; Coley & Barone 1996), so changes in these traits may have a significant impact on the subsequent feeding behaviour of each species. Pruning, for example, often improves the quality of browse for ungulates (du Toit *et al.* 1990), thereby attracting further browsing. Bergström *et al.* (2000) found that browsing by ungulates increased on previously clipped *Combretum apiculatum* trees and similarly, elephants tend to prefer mopane trees previously damaged by elephants, as the coppicing response provides a greater availability of their preferred branch size (Smallie & O'Connor 2000). Pruning also tends to increase the susceptibility of plants to insect herbivores, as regrowth often improves host suitability. Danell and Huss-Danell (1985), for example, found that the density of insect herbivores on moderately browsed birch trees was greater than on slightly browsed trees, due to the increase in leaf size and nitrogen content. Similarly, Martinsen *et al*. (1998) found an increase in leaf beetles on cottonwoods cut down by beavers, as coppice growth had a higher nutritional value and lower toughness. Pruning may, however, also have a negative influence on insect herbivory. For example, elk (*Cervus canadensis*) browsing on aspen (*Populus tremuloides*) was found to have a negative effect on the distribution and abundance of sawflies (*Phyllocolpa bozemanii*), due to a decrease in leaf size after browsing (Bailey & Whitham 2003).

In the absence of high elephant utilization of mopane trees, oviposition by mopane moths is primarily dependant upon tree size, with egg mass abundance increasing with increasing tree size (see Chapter 5). Elephant utilization, however, has the potential of significantly reducing tree size, and hence oviposition, as stem breakage can reduce a plants' biomass by 50-75% (Smallie & O'Connor 2000). On the other hand, pruning by elephants also results in a significant increase in leaf size and shoot length, which, according to other insect-host studies, may then have a positive influence on mopane moth oviposition. Similarly, caterpillar defoliation could have a negative influence on browsing by elephants through the resultant smaller shoot and leaf size (i.e. less biomass available) or alternatively, a positive influence due to the higher nutritional value of the leaves.

Unfortunately, it was beyond the scope of this project to look at the interaction between elephants and mopane caterpillars in both directions, and hence only the effect of elephants on mopane caterpillars was considered here. The aim of this study was therefore to investigate whether mopane caterpillars are influenced by elephants indirectly through their shared food resource, mopane. I tested for three alternative scenarios by which elephant impact on mopane trees could influence the oviposition behaviour of mopane moths: (1) a negative influence, due to reduced tree size; (2) no influence at all; (3) a positive influence, due to increased leaf size and nutritional value of resprouting mopane foliage.

6.2 Methods

The study was carried out within mopane woodland in the Shingwedzi area of the Kruger National Park, South Africa (see Chapter 2 for details).

In December 2003, when egg laying by mopane moths was complete and caterpillars were beginning to emerge (i.e. defoliation was still minimal), 14 transects of 50 x 5 m were set out within the mopane woodland surrounding Shingwedzi. Seven transects were situated in areas of heavy elephant utilization (HE), and the other seven were partners to these, in nearby areas of low elephant utilization (LE). Pairing of transects minimised the effects of spatial variability in rainfall and soil. HE transects were generally situated near man-made water holes and LE transects were chosen to resemble the corresponding HE areas (judged visually), in tree density and population structure (number and size of stems). While trees in the HE transects are also likely to have been more heavily utilised by other mammalian species (due to the close proximity to water), elephants would still have been largely responsible for any branch breakage, which is of primary concern here.

6.2.1 Area description

To quantitatively describe each area, the following information was recorded for all mopane trees within each transect: live tree height (m), basal stem diameter (cm), canopy width at the widest point (m), canopy height (m) and elephant utilization. An index of canopy volume was calculated for each tree as the volume of a cylinder, using canopy height and width values. For each transect, mean tree height, stem diameter and total canopy volume/ha were then determined. Tree density/ha was also calculated, by counting the total number of trees within the transect. Additionally, mature leaves were collected randomly from around the canopies of at least 5 trees per transect. Leaves from each transect were combined to assess the overall nutritional value of foliage in the area. Protein, tannin and total polyphenolic content was determined (see Chapter 3 for details), as these chemical characteristics commonly influence herbivory (Zucker 1983; Cooper & Owen-Smith 1985; Cooper *et al*. 1988; Coley & Barone 1996; Villalba *et al*. 2002).

Using data from the Venetia-Limpopo Nature Reserve (Venetia), where elephant impact on mopane is still relatively low (see Chapters 2 & 3), tree height and the diameter of the largest living stem per tree were found to be significantly correlated for mopane (Spearman-Rank, $P < 0.01$). The diameter of the largest stem may therefore be used as an indicator of a trees' potential height, without the negative effect of elephant utilisation. Hence, to assess whether LE and HE transect pairs would be similar in population structure (i.e. potential tree height), mean largest living stem diameters were compared.

6.2.2 Utilisation by elephants

The degree of elephant utilisation was measured for each tree as the proportion of canopy biomass removed by elephants, rated on the following seven point scale: 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-90%, 6 = 91-99%, 7 = 100% (Walker 1976). Utilization was categorised according to the type of damage, namely stem, branch or foliage, and the proportion of biomass removed was visually estimated from an imaginary intact plant. This was judged by the diameter of the broken stems/branches compared to those remaining, or the by the area of canopy without leaves. Additionally, the age of utilization was recorded, either as old, medium or new. New damage included that which occurred since the previous rainy season, and was characterised by the yellowish appearance of bark from freshly broken branches (Ben-Shahar 1993). Medium age damage was grey in colour with 'fairly young' coppiced branches around the damaged section (one to two years growth, estimated from personal observation after known age damage). Old damage was grey and weathered looking, with older coppiced branches.

To compare the degree of elephant utilization on trees, an index of utilization was calculated for each tree. Firstly, the accumulative impact was determined by adding the midpoints of relevant utilization classes (e.g. from medium and new browsing events; MacGregor & O'Connor 2004). Because canopy removal estimates were made according to the expected canopy volume at the time of browsing, accumulative values could exceed

100%. Therefore, to account for this, index values were calculated by giving the highest accumulative value a value of one, and all other values were worked out relative to this, on a scale of 0-1. Using these index values, the mean degree of elephant utilisation was calculated for each transect.

6.2.3 Oviposition by mopane moths

Within each transect, all trees with mopane egg masses from the current season were identified as host trees. Egg mass abundance per tree was recorded on a scale of 0-3, where 0 = 0 egg masses, 1 = 1 egg mass, 2 = 2 egg masses and 3 = 3 or more egg masses. Two measures of oviposition were calculated for each transect, firstly as the percentage of trees with egg masses (i.e. percentage of host trees) and secondly, as the number of egg masses/ha. Count estimates are expected to be fairly accurate, as most trees were small enough to search thoroughly and only 6 out of 69 trees had a score of 3.

To determine whether oviposition in an area is influenced by elephant utilization, the percentage of host trees and egg mass density in HE transects were compared to that in the corresponding LE transects. Then, to determine what elephant induced changes may influence oviposition behaviour, the percentage of host trees and egg mass density values were compared to the following descriptive variables for each transect: tree density (for all trees and only trees > 3 m), canopy volume/ha, mean shoot length, mean leaf length and mean leaf nutritional value (see Chapter 3 for data collection method details).

In Venetia, where elephant utilization was low, the number of egg masses per tree was positively related to tree size (Chapter 5). Therefore, the effect of tree size (which may be negatively affected by elephant utilization) on host preference was specifically investigated here too. Data from all 14 transects could not simply be combined, however, as the availability of tree sizes, and therefore host preference, could have varied between transects. Hence, transects were first made comparable by calculating relative size classes (see Fig. 6.1). To do so, the mean height of all trees within each transect was first determined (mean 1). All trees with a height above and below this mean value were then grouped separately, and the mean height of each of these two groups was calculated (mean 2 and 3). This resulted in three mean values, dividing tree height into four classes, namely: (a) small, (b) small-medium, (c) medium-large and (d) large. Canopy volume classes were calculated in the same way. To test for oviposition preference between classes, the total number of egg masses on trees within each height and canopy class was determined (for all transects combined) and compared to the number expected. Both tree height and canopy volume were used as measures of tree size, as even though one would expect the two to be autocorrelated, variability in canopy size tends to increase with tree size in mopane (see Appendix D). The expected number off egg masses was calculated both according to the number of trees within each canopy volume and height class, and the total canopy volume available within each canopy class.

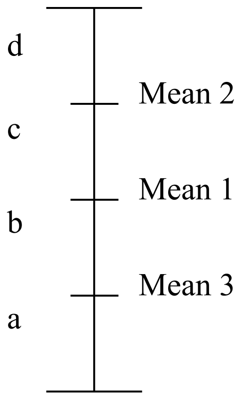

Figure 6.1 A graphic representation of how tree height and canopy volume classes (a-d) were calculated.

6.2.4 Statistical analyses

Wilcoxon matched-pairs tests were used to test for significant differences in (1) elephant utilization, (2) percentage host trees, (3) egg mass density, (4) tree density, (5) potential tree size, (6) leaf size, (7) shoot length and (8) foliar nutritional value between HE and LE areas.

Chi-squared goodness-of-fit tests were used to determine whether tree height or canopy volume classes were used disproportionately to their availability, both in terms of tree number and total canopy volume (Zar 1999). Where necessary, Bonferroni confidence intervals were used to determine which classes were preferred or avoided (Miller 1966; Neu *et al*. 1974; Byers & Steinhorst 1984).

6.3 Results

Designation of HE and LE areas was confirmed, as both the percentage of trees utilized by elephants, and the degree of elephant utilization per tree were higher in HE than LE areas (Table 6.1). In contrast, the percentage of trees with egg masses was significantly greater in LE transects, as was the egg mass density (Table 6.1, Fig. 6.2). There is therefore a negative association between mopane caterpillar abundance and elephant utilization of mopane trees within an area.

Total tree density did not differ between HE and LE areas, yet the density of tall trees (> 3 m) in LE areas was significantly greater than in HE areas. There was, however, no significant difference in the mean ‘largest stem diameter’. The reduced abundance of tall trees was therefore most likely due to elephant utilization, as the tree structure (tree height) of transect pairs was potentially the same. Similarly, the total canopy volume/ha was significantly greater in LE areas (Table 6.1).

Table 6.1 The mean ± SE values for variables describing areas of mopane woodland with high and low elephant impact, and the presence of mopane moth egg masses within these areas. The relationship between areas is also shown for each variable (Wilcoxon matched-pairs tests).

Variable	High elephant impact		Low elephant impact	*P* - value
Utilization				
% trees utilized by elephant	95.7 ± 1.58	>	60.6 ± 11.2	0.02*
Degree of elephant impact (index)	0.45 ± 0.06	>	0.16 ± 0.03	0.02*
% host trees (i.e. with eggs)	11.2 ± 2.77	<	22.9 ± 5.31	0.05*
Number of egg masses/ha	137 ± 35.8	<	440 ± 104	0.03*
Area description				
Largest stem diameter/tree (cm)	7.08 ± 1.09	=	7.09 ± 0.75	0.74
Total tree density/ha	982 ± 111	=	1411 ± 180	0.12
Density of trees > 3 m high	117 ± 37.9	<	394 ± 69.5	0.04*
Total canopy volume/ha (m^3)	7804 ± 797	<	14579 ± 2781	0.04*
Shoot length (cm)	115 ± 8.66	>	89.0 ± 12.6	0.04*
Leaf length (mm)	59.0 ± 1.47	=	56.6 ± 1.41	0.40
Tannin/protein ratio	0.48 ± 0.02	<	0.55 ± 0.02	0.02*
Total polyphenols (mg/g)	57.1 ± 1.69	=	59.3 ± 1.72	0.50

* Indicates significant differences at the 5% level

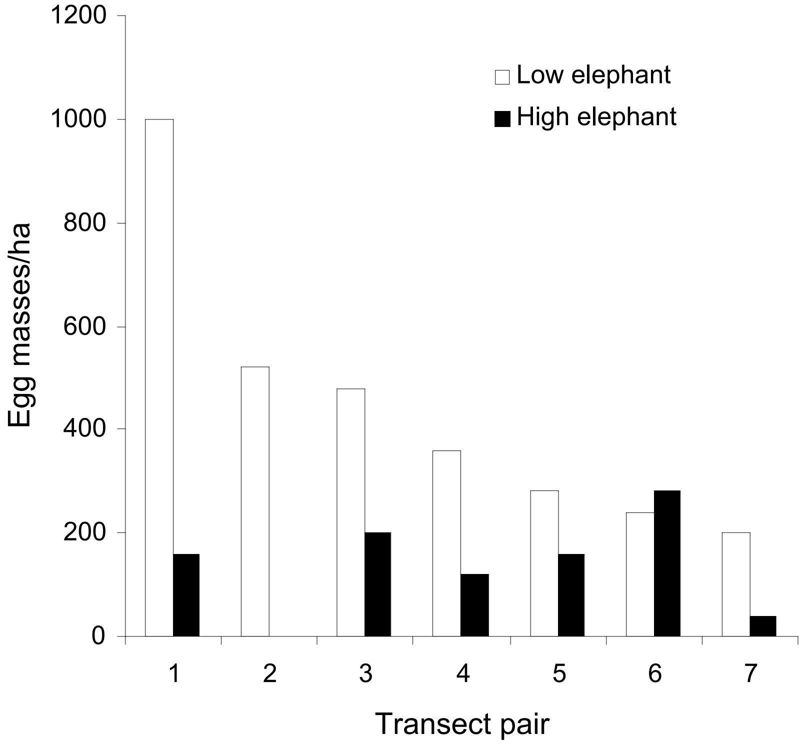

Figure 6.2 The density of mopane moth egg masses in areas of mopane woodland with high and low elephant impact (Wilcoxon matched-pairs test, $P < 0.05$).

As expected for pruned trees, shoot length was significantly longer in areas of high elephant utilization, yet unlike previous results (see Chapter 3), leaf length did not differ. Additionally, the total polyphenolic content of leaves was no different in HE and LE areas, yet the tannin:protein ratio was significantly lower in HE areas. This increase in foliar nutritional value with high levels of elephant impact also differs from results of my study in Venetia, where no change was observed (Chapter 3). The reduction in tannins and the lack of increase in leaf size is most likely an indication of the higher degree of stress in Kruger trees, which would have been subjected to elephant utilization for a more prolonged period compared to Venetia, where elephants were only reintroduced in 1991 after a minimal 50-year absence.

Considering only those variables that did differ between HE and LE transects, egg mass density was negatively related to shoot length and leaf nutritional value, yet was positively related to the density of tall trees and the total canopy volume/ha (Fig. 6.3). Elephant utilization therefore appears to have a negative impact on the density of mopane caterpillars in an area by reducing the number of tall trees and the total canopy volume/ha.

Similarly, based on tree number, ovipositing females were found to prefer the tallest trees and those trees with the largest canopy volume within an area, while shorter trees and trees with a smaller canopy volume were avoided (Table 6.2). When considering the actual resource availability within each canopy size class, however, no class was utilised more or less than expected in relation to its availability ($\chi^2 = 1.97$, df = 3, $P = 0.579$).

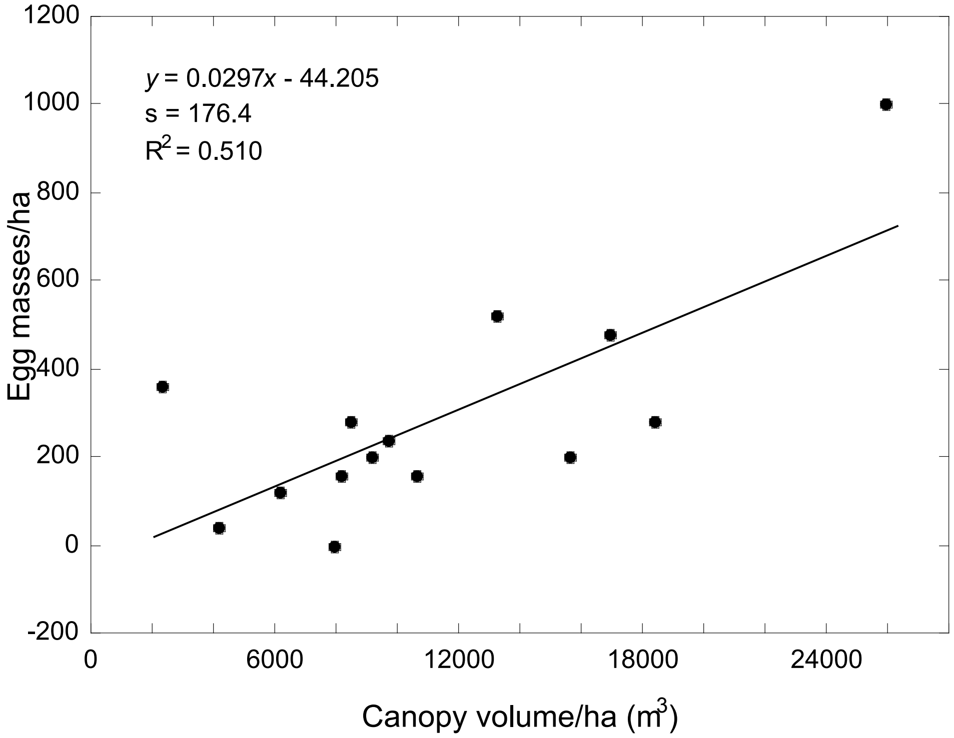

Figure 6.3 The relationship between the number of mopane moth egg masses/ha and mopane tree canopy volume/ha in mopane woodland ($F_{1,12} = 14.5$, $P < 0.01$, $R^2_{adj} = 0.510$).

Table 6.2 The selection of different size mopane trees by ovipositing mopane moth females, based on the number of trees within each size class and using canopy volume and tree height as two measures of tree size.

Class	Proportion of trees	Observed egg masses	Proportion of observed egg masses	Bonferroni intervals	Selection[#]
Canopy volume					
Small	0.437	9	0.090	0.018<p<0.162	-
Small-medium	0.269	17	0.170	0.076<p<0.264	-
Medium-large	0.173	30	0.300	0.185<p<0.415	+
Large	0.121	44	0.440	0.316<p<0.564	+
Tree height					
Small	0.283	6	0.060	0.001<p<0.119	-
Small-medium	0.296	13	0.130	0.046<p<0.214	-
Medium-large	0.234	15	0.150	0.061<p<0.239	0
Large	0.187	66	0.660	0.542<p<0.778	+

Symbols indicate classes used significantly more (+), less than (-), or equal to their availability (0), determined by Bonferroni confidence intervals.

6.4 Discussion

This study found that a high level of elephant utilization on mopane trees has a negative impact on the density of *Imbrasia belina* egg masses in an area, even though trees previously browsed by elephants had longer shoots and were of a higher nutritional value than unutilised trees. This negative relationship with leaf nutritional value found here may suggest that oviposition is influenced by leaf quality, yet results from previous work showed an opposite trend (Styles & Skinner 1996; Chapter 5), thereby indicating this not to be the case. A result that is consistent for all three studies, however, is the decrease in egg mass density with decreasing tall tree density (associated with high elephant impact). This therefore confirms tree size, measured both as tree height and canopy volume, to be the primary determining factor of mopane moth oviposition behaviour. When considering resource availability on larger trees versus egg abundance, however, no preference for larger trees was found. The greater utilization of large trees was therefore probably due to the greater chance of moths intercepting the larger bodies, rather than actual preference (but see discussion in Chapter 5).

The lack of preference for previously browsed plants is unlike results from many other studies on insect-mammal herbivore interactions, where plants tend to become more vulnerable to insect damage after pruning (Danell & Huss Danell 1985). Most previous studies have, however, only considered species that are likely to be dependant upon resource quality for larval performance, due to their feeding behaviour and relatively low population densities i.e. minimal resource competition. Densities of sap sucking (Schowalter & Ganio 1999; Ostrow *et al*. 2002) and galling (e.g. sawflies, Hjältén & Price 1996) species have, for example, been found to increase with increasing leaf/shoot size and/or nutritional value. Some studies on leaf-chewing species have also shown this trend, such as that by Martinsen *et al*. (1998) who found an increase in leaf beetle densities on cottonwoods previously felled by beavers. Where mopane moths differ to most other species studied, however, is that its' larvae are relatively immobile (confined to one or a few trees) and tend to reach outbreak proportions. During such outbreaks the population may become density dependent due to resource competition, thereby making resource quantity, rather than quality, the primary limiting factor for the species. Interestingly, Schowalter and Ganio (1999) found a similar trend of reduced defoliator insect numbers in recovering hurricane-damaged forest patches, which is likely to resemble elephant damage in its severity. The species of insect was not given, however, and unfortunately no other studies on the effect of pruning on an outbreak defoliator species could be found for comparative purposes.

According to Vance (1985), a number of possible mechanisms enable two competitors for one resource to coexist. These include: (1) the use of different body parts, (2) the use of different growing seasons and (3) spatial subdivision in the environment. Under 'normal' conditions, when elephant densities are not too high, elephants and mopane caterpillars fulfil all three of these criteria, and therefore display a degree of resource partitioning. Firstly, as already mentioned, the two species mostly utilize different plant parts. Mopane caterpillars feed only on the leaf blades and elephants feed mainly on branches (Smallie & O'Connor 2000). Secondly, the majority of branch breakage by elephants takes place in the dry season (Lewis 1986), while mopane caterpillar defoliation occurs in the summer. Elephants do also feed on leaves during this time, but the impact on individual trees is minimal compared to branch breakage. Finally, there is also a spatial separation in their use of mopane trees, both at the habitat and individual tree level. In Venetia, for example, mopane moths were found to prefer the tall riverine trees and egg masses were more abundant on tall trees (Chapter 5), while elephants preferred shorter trees of < 2 m (Smallie & O'Connor 2000) and therefore concentrated their feeding in the woodland or shrubland habitats. This minimal usage of large trees by elephants was even found in the relatively high elephant density areas of northern Botswana, as even though 43% of mopane trees were affected by elephants, the density of large trees remained largely unchanged (Ben-Shahar 1998).

The use of larger trees by elephants is, however, known to increase when smaller size classes are depleted (Lewis 1991). Such a situation could be episodic, in association with events such as fire, frost or drought (MacGregor & O'Connor 2002), which may cause high mortality of trees up to 3m in height (Chafota 1998). Alternatively, the severe impact on canopy trees could be in localised areas (Ben-Shahar 1993), caused by a high concentration of animals around permanent water sources (see Fig. 6.4), or due to elephant movements being restricted by physical barriers, such as game fencing (Cumming *et al.* 1997). Local population densities may also increase 'unnaturally' in open systems, in response to habitat loss due to an expanding human population and development (Ben-Shahar 1996). Increased utilization of tall trees by elephant, and the subsequent decline in the mopane caterpillar population, may therefore occur naturally, but has the potential to be greatly enhanced by mans' activities.

While no actual preference for large canopied trees was detected, the direct relationship between egg mass number and tree height would mean that the larger the tree felled by an elephant, the greater the impact on the mopane caterpillar population. The felling of a tall tree would not only have an immediate effect on resource availability for mopane caterpillars, but also a long term effect, as felled trees tend to be continuously browsing by elephants (Smallie & O'Connor 2000), keeping their height suppressed (termed 'hedging', Styles 1993). Additionally, hedging could have a long-term impact on tree density (and hence resource availability) by preventing the recruitment of trees into the larger, reproductive size classes (see Chapter 4), and through increased tree mortality

Figure 6.4 Mopane woodland heavily impacted by elephants, resulting in a double-tiered woodland structure consisting of tall, intact trees and coppicing damaged trees (the photo was taken in Chobe National Park, Botswana).

from the continual browsing pressure. Any negative impact of elephant utilization on tree height could therefore affect mopane caterpillar abundance indirectly in many ways, for long periods of time.

According to Bailey and Whitham (2003), the interaction between common and/or keystone species is likely to result in community-wide consequences. In their study, they showed how browsing by elk had a negative affect on the distribution and abundance of sawflies, which resulted in up to a 33% reduction in the arthropod community associated with sawflies. Additionally, browsing had a negative impact on bird foraging on galls, by altering the density of their prey. Beavers, considered as a keystone species, have also been found to affect the distribution of a common leaf-feeding beetle, which in turn affects the diversity of other taxa (Martinsen *et al*. 1998). Likewise, *Imbrasia belina* are also preyed upon by a diverse array of species. Pupae are readily dug up by jackals (*Canis mesomelas*), bat-eared foxes (*Otocyon megalotis*), warthogs (*Phacochoerus aethiopicus*) and aardvarks (*Orycteropus afer*), for example, while, larvae are eaten by mammals (mainly baboons, *Papio ursinus* and vervet monkeys, *Cercopithecus aethiops*), birds (34 species were documented by Styles 1995), and invertebrates (e.g. spiders, ants, and mantids; Styles & Skinner 1996). Moths are also taken by birds and spiders, as well as by bats while active at night (Hill & Smith 1984). The negative impact of elephants on *I. belina* could therefore also have an indirect effect on a great diversity of species from different trophic levels.

One species in particular likely to be affected by any decrease in the mopane caterpillar population, is man. The larvae forms an important food source for rural communities by providing a vital source of protein (65% of dry mass), especially during lean periods. Harvested in large numbers, it has become an important trading commodity.

In Botswana, for example, the mopane industry earns the country an estimated R46 million annually (Potgieter *et al.* 2001).

In conclusion, elephants and mopane caterpillars usually display resource partitioning within mopane woodland, yet under conditions of high elephant impact, mopane caterpillar abundance decreases. These two members of the mopane browsing guild do therefore interact indirectly through their shared resource. The negative effect of elephant utilization on mopane caterpillar abundance appears to operate through the decrease in density of tall mopane trees. This association raises implications for the conservation of mopane caterpillars as a protein source for rural people, as any factor increasing elephant impacts on mopane trees (e.g. fences and waterholes) will also influence mopane caterpillar abundance, and also probably the subsequent biodiversity in the area.

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CHAPTER 7

ELEPHANT BROWSING, CATERPILLAR DEFOLIATION AND FLUCTUATING ASYMMETRY IN *COLOPHOSPERMUM MOPANE* LEAVES

7.1 Introduction

Plants respond to herbivores in a variety of ways, such as by changing the allocation of resources between defence, regrowth and reproduction, or by increasing photosynthesis in the undamaged tissue (Bryant *et al.* 1983; Herms & Mattson 1992; Danell *et al.* 1994; Strauss & Agrawal 1999; Bellingham & Sparrow 2000; Strauss *et al.* 2002; Díaz *et al.* 2003). Additionally, these individual-based, short-term responses are variable within a species and may depend on factors such as the type, timing and degree of damage (Maschinski & Whitham 1989; Lennartsson *et al.* 1998; Tiffin 2002). Measuring the degree of impact of herbivory on plant fitness is therefore not simple and frequently used indicators of stress include: decreases in productivity (Boyer 1982); changes in physiological parameters such as leaf water deficit (Griffiths & Parry 2002); and plant biochemistry (Louda & Collinge 1992). Factors such as leaf size and shoot growth are also commonly used to evaluate plant vigour. Most of these characteristics, however, often have a low sensitivity or specificity of responses and are therefore of limited use. For example, compensatory responses could mask the more long term detrimental effects of herbivory, as damaged plants could initially perform as well as or better than non-damaged controls (Agrawal 2000).

Often considered a more reliable indicator of stress is the measure of developmental instability (DI). Fluctuating asymmetry (FA), which reflects small random deviations from symmetry in otherwise bilaterally symmetrical characters (Palmer & Strobeck 1986), is the most commonly used measure of DI. Since the development of the right and left sides of a bilaterally symmetrical morphological character are controlled by the same genes, the degree of FA is thought to represent a compromised ability to control developmental processes under given environmental conditions (Møller 1995; Møller & de Lope 1998). A variety of stresses can cause increases in FA, including extreme climatic conditions (Valkama & Kozlov 2001), restricted nutrition (Lappalainen *et al.* 2000), toxicity (Kozlov *et al.* 1996) and intra- and interspecific competition (Rettig *et al.* 1997), as well as genetic factors such as mutation, inbreeding and hybridisation (Møller 1996; Wilsey *et al.* 1998; Hochwender & Fritz 1999; Møller & Shykoff 1999). Measures of DI are therefore sensitive indicators of developmental performance of organisms and as a result, FA is considered a useful and objective tool for measuring stress levels in both plants and animals, where in most cases an increase in asymmetry is directly related to a decrease in growth, fecundity and survival (Møller 1997; Møller 1999). Díaz *et al.* (2004), for example, showed that developmental instability caused by herbivory had possible consequences for plant fitness, as leaf FA increased with herbivory and FA was inversely related to reproduction. Additionally, studies have found leaf FA to increase after insect herbivory, although no change in leaf size was observed (Zvereva *et al.* 1997; Møller & de Lope 1998). Asymmetry level therefore provides a sensitive measure of plant performance, even when plants respond to herbivory with compensatory growth.

Developmental stress can lead to physical as well as chemical changes in leaves. Møller (1995) suggests that in plants, if resource allocation to developmental control competes with allocation to the production of defensive compounds, the ability to develop symmetrical characters may then be linked to the ability to produce secondary compounds. Alternatively, the genes affecting chemical defence may be polyphonic, and also affect the level of FA. Additionally, developmental instability may be associated with higher leaf nutritional value due to accelerated growth in response to browsing, as found by Martel *et al*. (1999). In plants, browsing that removes apical dominance tends to induce the production of larger and more nutritious leaves (Lehtilä *et al*. 2000). This is possibly due to the disturbance of hormonal control in the plant (Honkanen & Haukioja 1998), thereby resulting in less regulated developmental processes and an increase in resource flow to previously suppressed meristems, which in turn could cause developmental errors (Martel *et al.* 1999). Whether due to stress or accelerated growth, developmental instability is therefore often associated with increased leaf nutritional value for herbivores. Consequently, plants with a higher degree of FA are also often more susceptible to further herbivory. For example, Møller (1995) found the increased asymmetry in elm leaves (*Ulmus glabra*) caused by a fertilizer treatment gave rise to an increased abundance of leaf miners (*Rhynchaenus rufus*).

While the majority of studies on developmental instability have previously tended to focus on animals, more recently, FA has been applied more frequently to plants and plant-herbivore systems (reviewed by Møller & Shykoff 1999). This is perhaps because plants are such suitable organisms for studying developmental instability, due to their modular structure, which results in repeated structures that reflect developmental performance (Freeman *et al.* 1993). Additionally, it has been suggested that due to the extreme phenotypic plasticity in plants (reviewed by Schlichting 1986), the regulatory genes that control developmental homeostasis and symmetrical morphogenesis may be even more important in plants than animals (Møller 1995).

In perennial woody plants, the character most commonly used to measure stress is foliar FA (e.g. Zvereva *et al*. 1997; Martel *et al.* 1999). The leaves of *Colophospermum mopane* trees (commonly known as 'mopane', see details in Chapter 2) are particularly suitable for such measurements, as they are pinnate, with two large leaflets (Fig. 7.1). Mopane trees are utilised by two key browsers, namely mopane caterpillars (*Imbrasia* *belina*) and African elephants (*Loxodonta africana*), each of which inflicts a different type of damage while feeding. While mopane caterpillars defoliate plants by removing just the leaf blades, elephants have a pruning effect by breaking off branches or the main stem (Lewis 1991; Smallie & O'Connor 2000). Mopane trees are known to respond differently to each of these damage types, by producing smaller leaves after defoliation, but larger leaves after pruning (Chapter 3). However, due to these differences in response, it is not clear which browsing type has the greater (if any) negative effect on plant fitness. Since foliar developmental stress is expected to occur in mopane trees, in association with the observed changes in growth, comparing the level of FA in defoliated versus pruned trees might therefore prove to be a useful indicator as to which browsing type has the greater stressing affect. With such knowledge, we could significantly improve our understanding of the more long-term impact of herbivory, as the comparative stress on a single species has not been looked at in this manner before.

Additionally, by knowing the degree of stress caused by browsing, we can understand a plants' vulnerability to further herbivory, specifically by mopane caterpillars in this case. Although no significant relationship between foliar nutritional value and oviposition site choice by mopane moths was found in a previous study (Chapters 5 & 6), this could have been due to an inappropriate choice of compounds being considered (proteins, tannins and total polyphenols). Mopane's complex chemical make-up would, however, make it difficult to choose the appropriate compounds. Therefore, if FA and leaf chemistry are related in mopane trees, FA could indicate less specific differences in secondary compounds. The level of FA in host and non-host trees could then also be a way of investigating whether host choice by ovipositing females is related to leaf chemistry. Firstly, however, it should at least be determined whether FA is an indicator of mopane leaf 'quality' for those chemical compounds measured.

The aim of this study was therefore to investigate the effects of browsing on the developmental instability (i.e. stress) of mopane trees, and the influence of plant stress on the susceptibility to defoliation by mopane caterpillars. The following main questions were addressed: (1) Does defoliation by mopane caterpillars or pruning by elephants affect foliar FA in mopane trees and if so, which has the greater impact? (2) Are foliar FA and leaf chemistry related in mopane? (3) Does foliar FA in host trees differ to that in neighbouring non-host trees?

7.2 Methods

The study was carried out within the Venetia-Limpopo Nature Reserve (i.e. Venetia, see Chapter 2 for details).

7.2.1 Response to browsing

Mopane trees were subjected to various simulated elephant and mopane caterpillar browsing treatments, as described in Chapter 3 (the same trees were used here). The sample size per treatment was initially 15 trees, yet a number of trees were defoliated by a species of puss moth larva (Family: Notodontidae, Order: *Epicerura*; Picker *et al*. 2002) during the study period and therefore had to be excluded from the study. Sample sizes therefore ranged from 10 – 15 trees per treatment and treatments were as follows (dates indicate time of application): (1) Elephant 2002 = E'02; (2) Elephant 2003 = E'03; (3) Caterpillar November 2002 = CN'02; (4) Caterpillar February 2003 = CF'03; (5) Caterpillar November 2002 and February 2003 = CN&F; (6) Caterpillar November 2003 = CN'03; (7) Control = Con.

Treatments were applied when natural utilization typically takes place i.e. elephant browsing in August (the end of the dry season; Lewis 1986) and caterpillar browsing in November and February (during the two brood sessions). The variation in treatments enabled investigation into the comparative effects of early versus late-season defoliation and repeated defoliations, as well as current versus following-season (delayed) responses. In mid-February 2004, 15 mature, undamaged leaves were collected randomly from all sides of the canopy of each tree, between a height of 1-2 m. On the same day of collection, leaf asymmetry was recorded for two morphological traits, namely leaf length and width. Leaf length was measured for each leaflet from the base (where the two leaflets join) to the apex. Each leaflet width was measured at the midpoint between the base and tip, perpendicular to the line joining these points (Fig. 7.1). Measurements were taken with a transparent ruler to the nearest 0.5 mm. To test for measurement error, a random sample of 90 leaves were measured a second time on the same day, with no reference to prior measurements.

Due to the questionable validity of simulated herbivory mimicking natural damage (Baldwin 1990; Tiffin & Inouye 2000; Hjältén 2004), naturally defoliated and elephant-pruned trees used to measure regrowth in Chapter 3 were also sampled here. Defoliation by mopane caterpillars took place in December/January 2004 and leaves were collected from all trees in early March 2004 (15 defoliated and control trees, 13 pruned and control trees).

Furthermore, the effect of repeated natural defoliation within the same season was investigated. In November 2003 an area of mopane trees was completely defoliated by a species of puss moth larva (the same species as previously mentioned) that had a similar method of defoliation as mopane caterpillars i.e. entire leaf blades were removed. Fifteen of these trees were marked and in mid-December 2003 (when trees had re-flushed but were not yet being consumed by mopane caterpillars) 15 leaves were collected from each tree. In December/January the same trees were defoliated again, but by mopane

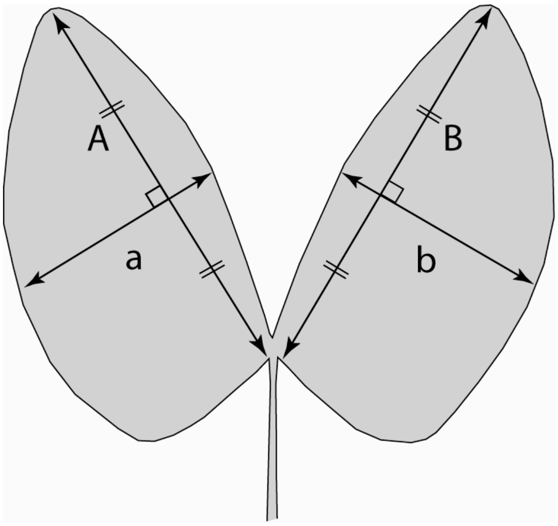

Figure 7.1 A diagram of a mopane leaf, showing the measurements taken to determine fluctuating asymmetry. Asymmetry (FA) in: length = (A-B) and width = (a-b). Absolute asymmetry_FA_in: length = _A-B_and width = _a-b_.

caterpillars. In early February, after the same re-growth time as the first sampling effort allowed, leaves were once again collected from the 15 trees. All leaves were measured as described earlier.

For all trees sampled, additional mature leaves were collected randomly from around the canopy of each tree to determine the foliar chemical composition. Leaves were first air dried in brown paper bags and later oven dried at 50°C for at least four days. The protein, condensed tannin and total polyphenolic content was then determined (see Chapter 3 for method details).

7.2.2 Host selection

Foliar FA of trees with egg masses (host trees) was compared to neighbouring conspecific trees without egg masses (non-host trees). The same 30 tree pairs identified in Chapter 5 were used here (15 pairs in riverine and 15 pairs in woodland habitat). Fifteen leaves from each tree were collected and measured in November 2002, just after egg laying by mopane moths was complete. Leaves were also collected for chemical analysis.

7.2.3 Statistical procedure

To investigate whether leaf length and width demonstrated fluctuating asymmetry, it was tested whether signed right-minus-left leaf length and width values deviated from a normal distribution (Kolmogorov-Smirnov test) with a mean value of zero (t-test, Palmer & Strobeck 1986; Swaddle *et al.* 1994), for all leaves combined ($n = 3374$). Significant deviations would indicate that the characters demonstrated directional asymmetry (mean values deviated from zero) or anti-symmetry (frequency distributions deviated from normal distribution).

According to Mosimann and Campbell (1988), multiplicative errors occur whenever growth is active, i.e. when growth involves the addition of tissue to that which is already present. Leaves of plants grow in such a manner, resulting in possible increases in asymmetry with increasing leaf size. As recommended by Graham *et al.* (2003), all data were therefore transformed using _log R – log L_ to effectively remove the effects of size scaling. Absolute leaf length and width asymmetry (_FA_) is defined as the unsigned difference between the right and left leaflet of a particular leaf. The mean _FA_ was calculated for each tree.

The significance of FA relative to measurement error was checked using a two-way factorial ANOVA for each trait, with the factors being 'individual leaf' and 'side' (left or right), and the double measurements on each side considered as replicates (Bañuelos *et al.* 2004).

To assess the degree of intra-tree variability in FA in browsed and non-browsed trees, the coefficient of variation (CV) was calculated for each naturally defoliated and pruned tree, as well as control trees. Additionally, CV values for trees from the simulated treatments that responded most to pruning or defoliation (with largest or smallest leaves), were calculated i.e. trees from CN&F, E'03 and Control treatments. Mann-Whitney U tests were then used to test whether the degree of intra-tree variation in FA was different after natural defoliation or pruning, and a Kruskal-Wallis ANOVA was used to test for differences between the simulated treatments.

A one-way ANOVA was used to test for differences in leaf asymmetry after the various simulated elephant and caterpillar treatments. Paired t-tests were used to test for differences between (1) naturally defoliated and non-defoliated trees, (2) naturally pruned and non-pruned tees, (3) trees naturally defoliated once and twice and (4) host and non-host trees. Data from woodland and riverine habitats were analysed separately when comparing host and non-host trees. Variation between woodland and riverine habitats was analysed using a Student's t-test, with all sampled trees (host and non-host) within each habitat type combined. Data were square-root transformed when not normally distributed (Zar 1999).

The relationship between leaf chemistry and FA was tested first within each experiment (simulated treatments, natural treatments and host selection transects), and then for all data combined. Only trees naturally defoliated twice were not included here, as leaves were very young when sampled and would not have been comparable chemically (Coley 1988). Simple regressions were used to test for relationships between FA and protein, tannin or total polyphenolic content within experiments, and a Spearman rank correlation was used when all data were combined, as the FA data was then not normally distributed.

7.3 Results

The frequency distribution of asymmetry in leaf length had a mean value of zero (t-test: $P > 0.05$), but was leptokurtically distributed (Kolmogorov-Smirnov test: $P < 0.01$; kurtosis = 3.96). Although Palmer (1994) recommends that only measures showing a normal distribution should be used in FA research, more recent work however, argues that whenever individual differences in developmental imprecision exist, asymmetry should be leptokurtically distributed (Leung & Forbes 1997; Gangestad & Thornhill 1999). Leaf length was therefore considered to exhibit FA.

The frequency distribution of asymmetry in leaf width, however, did not have a mean value equal to zero (t-test: $P < 0.05$). Instead, the distribution displayed a negative skew (-0.294 ± 0.042; i.e. directional asymmetry), where there was a constant bias for the left side to be wider than the right side. According to Palmer and Strobeck (1992), directional asymmetry does not have an ideal state that is definable *a priori*, making it impossible to separate asymmetry caused by developmental instability from that with a genetic basis. Leaf width could therefore not be used as an indicator of developmental instability and consequently, a composite FA index combining leaf length and width (as recommended by Leung *et al*. 2000), could not be used. Leaf length FA alone was therefore used for all analyses.

The interaction term (individual leaf x side) was significant for both length and width measurements ($F_{89, 89} = 160$, $P < 0.001$; $F_{89, 89} = 70$, $P < 0.001$ respectively), but the interaction (individual leaf x measure) was not significant for either trait (length: $F_{89, 89} = 1$, $P = 0.177$; width: $F_{89, 89} = 1$, $P < 0.060$). Between-sides variation (FA) was therefore significant, but no significant measurement error was detected.

No significant difference in FA was found among the simulated treatments (ANOVA: $F_{6,79} = 1.29$, $P = 0.274$) or between naturally browsed and non-browsed trees (caterpillar: $t = -0.253$, df = 14, $P = 0.804$; elephant: $t = 0.364$, df = 12, $P = 0.722$; paired t-tests). Additionally, FA on specific trees did not differ significantly after being defoliated once or twice (paired t-test: $t = 1.19$, df = 14, $P = 0.255$). Neither defoliation nor pruning therefore had an affect on mopane tree developmental instability (see Table 7.1 for details).

The intra-tree coefficient of variation (CV) in FA varied considerably between trees within the same treatment, even for control trees (Table 7.2). There was, however, no significant difference in CV values between natural (caterpillar: $Z = 0.353$, $P > 0.05$; elephant: $Z = 0.026$, $P = 0.980$; Mann-Whitney U tests) or simulated treatments (Kruskal-Wallis ANOVA: $\square^2 = 0.168$, df = 2, $P = 0.920$). Individual trees therefore differ in the degree of variability in FA in their leaves, but this is not dependant upon previous browsing.

Table 7.1 Foliar FA in mopane (means ± SE) regrowth after various browsing treatments[1] and for trees with and without mopane moth egg masses[2]. Estimates are from measures of leaf length, as mean values of the signed difference between right and left sides of a leaf (R-L), and as absolute values of the difference between left and right sides (absolute leaf FA).

	Leaf length (mm)	R-L leaf length (mm)	Absolute leaf FA
Simulated treatments:[1,_]			
Con	55.9 ± 0.67	0.57 ± 0.23	2.09 ± 0.14
CN&F	49.5 ± 0.88	0.01 ± 0.21	2.06 ± 0.21
CN’02	52.1 ± 1.18	0.16 ± 0.27	2.34 ± 0.20
CN’03	54.0 ± 0.49	0.13 ± 0.19	2.27 ± 0.15
E’02	61.6 ± 1.37	0.29 ± 0.26	2.40 ± 0.20
E’03	62.8 ± 1.16	0.10 ± 0.22	2.24 ± 0.15
CF’03	51.0 ± 0.61	0.03 ± 0.26	2.14 ± 0.17
Naturally utilized:[1]			
Caterpillar	45.4 ± 0.95	0.33 ± 0.14	1.90 ± 0.16
Caterpillar controls	57.3 ± 1.25	0.34 ± 0.22	2.36 ± 0.16
Elephant	62.6 ± 1.46	-0.31 ± 0.23	2.52 ± 0.18
Elephant controls	49.6 ± 0.75	0.15 ± 0.12	2.07 ± 0.15
Repeated defoliation:[1]			
After first	44.5 ± 0.82	-0.05 ± 0.16	1.93 ± 0.20
After second	39.5 ± 0.83	0.05 ± 0.16	1.46 ± 0.10
Host selection:[2]			
Woodland - host trees	45.6 ± 0.76	0.15 ± 0.17	1.88 ± 0.17
- non-host trees	45.0 ± 0.70	0.38 ± 0.18	1.53 ± 0.08
Riverine - host trees	55.9 ± 0.76	-0.12 ± 0.14	2.02 ± 0.11
- non-host trees	49.2 ± 0.58	0.03 ± 0.14	2.04 ± 0.14

_ Simulated treatment codes: Control = Con, Caterpillar November 2002 and February 2003 = CN&F, Caterpillar November 2002 = CN’02, Caterpillar November 2003 = CN’03, Elephant 2002 = E’02, Elephant 2003 = E’03, Caterpillar February 2003 = CF’03. Dates indicate time of application. See text for more details.

Table 7.2 The mean coefficient of variation (CV) in values of leaf FA within individual trees after natural and simulated herbivory.

Utilization	Mean CV	Min CV	Max CV
Simulated			
Control	95.9	72.9	135
Caterpillar	97.3	66.8	151
Elephant	84.4	58.8	113
Natural			
Caterpillar control	79.3	57.6	105
Caterpillar	87.3	44.0	177
Elephant control	85.4	47.4	133
Elephant	88.3	54.6	127

No significant difference in FA was found between host and non-host trees in the woodland or riverine habitats (paired t-tests: t = -1.40, df = 13, P = 0.186 and t = 1.62, df = 14, P = 0.128 respectively). Host tree selection by mopane moths was therefore not related to FA. Additionally, foliar FA in woodland and riverine habitats did not differ significantly (Student's t-test: t = -0.515, df = 56, P = 0.609).

Leaf chemistry was not related to FA when data from each experiment was considered separately (regressions: $P > 0.05$). For all data combined, however, FA was significantly positively correlated to protein and significantly negatively correlated to total polyphenolic and tannin content (Fig. 7.2). The relationship with tannin was strongest.

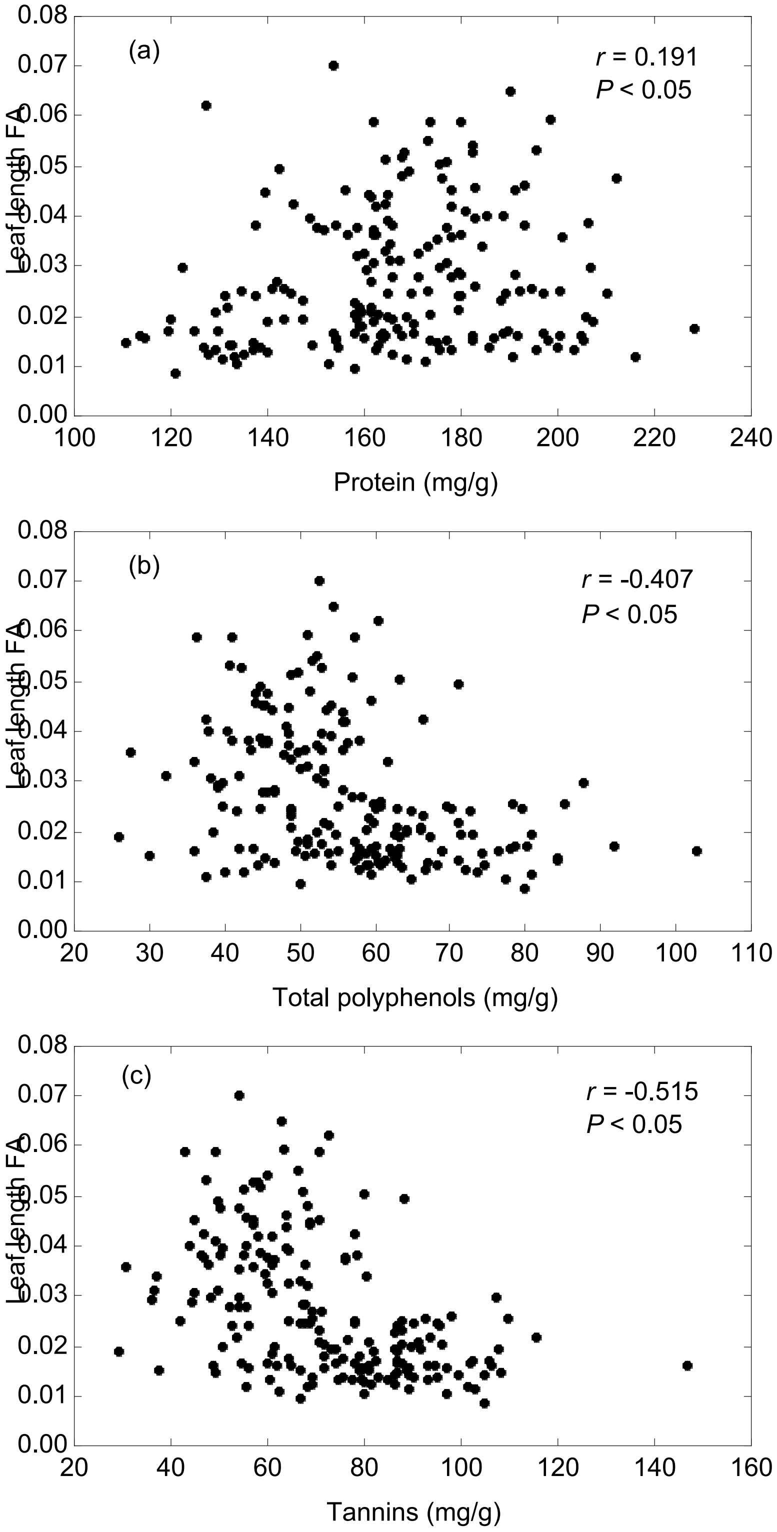

Figure 7.2 The relationship between mean fluctuating asymmetry in leaf length (_log R – log L_) and foliar (a) protein, (b) total polyphenolic and (c) tannin content in mopane trees, for all study trees combined.

7.4 Discussion

Mopane leaves showed fluctuating asymmetry in one of the two characters measured, namely leaf length. On a broad scale, foliar chemistry and FA were significantly correlated, with developmental instability increasing with leaf nutritional value. However, neither simulated nor natural defoliation by mopane caterpillars and pruning by elephants was found to affect the level of leaf FA. Similarly, host choice by ovipositing moths was not related to FA.

The lack of change in leaf FA after browsing is unlike results from numerous other studies, where an increase in FA was found to be the common response (Zvereva *et al*. 1997; Møller & de Lope 1998; Martel *et al*. 1999; Díaz *et al*. 2004). Considering the relationship between FA and leaf chemistry found in this study, it perhaps not surprising that no increase in developmental instability was detected in pruned trees, as there was also no change in leaf chemistry (see Chapter 3). Contrary to expectation, however, the level of FA did not increase with leaf size after pruning, which is unusual as rapid growth (which occurred after pruning, see Appendix B) is also known to cause developmental instability (Martel *et al*. 1999). This suggests that developmental instability in mopane trees is not determined by leaf growth rate, but rather by the plant's leaf chemistry, which is hence more closely related to plant stress.

With regards to leaf FA after defoliation, a similar lack of response has also been reported for other species, yet these findings tended to be either after a once-off defoliation event (Lappalainen *et al*. 2000) or from the first but not second regrowth event i.e. delayed response (Díaz *et al*. 2004). The level of FA in mopane, however, did not change after any of the simulated defoliation treatments, whether measured after repeated defoliations or as a delayed response the following season. As with pruning, here too it is not surprising that no change in FA was detected, as there were also no changes in leaf chemistry (see Chapter 3). Naturally defoliated trees, however, which were likely to have been defoliated repeatedly in the past, were found to have significantly smaller leaves and reduced chemical defences. This inability to produce as many secondary compounds, together with the retarded growth, suggests that the trees were under stress, yet still no changes in FA levels were recorded. A possible reason for the lack of relationship between leaf FA and the level of defensive chemicals could be that an increase in chemical defences is not the optimal allocation of resources for defoliated mopane, due to the severity and frequency of attack trees tend to experience (as discussed in Chapter 3; Doak 1992; Messina *et al.* 2002). The decrease in chemical defences could therefore be part of the plant's tolerance strategy, rather than a consequence of stress.

From these results mopane therefore appears to be extremely tolerant of herbivory compared to other species, as even though the level of herbivory (caterpillar: > 90% defoliation, elephant: 75% biomass removal) was equal to or greater than in studies where increases in FA have been reported (e.g. Zvereva *et al.* 1997; Martel *et al.* 1999; Zvereva & Kozlov 2001), no changes in FA were observed here.

According to Møller (1997) two possible reasons could explain the lack of relationship between herbivory and FA: (1) FA was measured with such large error that the true relationship disappeared and (2) the relationship may only exist under extreme environmental conditions. While the problem of measurement error is not valid here, as this was tested for and found not to be significant, the second point is, however, quite possibly an explanation. What suggests this is that the relationship between leaf chemistry and FA was only significant when trees from all experimental areas were considered together. Apart from a larger sample size, a greater variety of environmental conditions (e.g. soil chemistry, soil drainage, shading and competition) would have been represented here compared to when each area was considered separately, possibly including conditions extreme enough to cause stress in mopane trees (see variability in soil between habitats in Table C.2, Appendix C). Environmental conditions, rather than herbivory therefore appears to be the greater stressing agent for mopane trees, as has been reported for other species. White birch (*Betula pubescens*), for example, had increased levels in FA after changes in resource availability, but not defoliation (Lappalainen *et al*. 2000). Similarly, leaf FA in the willow species *Salix borealis* only increased in response to defoliation when grown in a polluted habitat (Zvereva & Kozlov 2001). Consequently, the stressing affect of pruning and defoliation on mopane trees may then only become apparent if measured in a variety of habitats, in particular where plants are already likely to be under stress (see Hawkes & Sullivan 2001).

For mopane, any stress caused by herbivory could also have been difficult to detect due to the high degree of intra-tree variability within an area, irrespective of previous utilisation. According to Møller and Shykoff (1999), it is common for species to show such inconsistency in their symmetry for certain characters. In the common fig (*Ficus carica*), for example, leaf FA within a tree was found to depend on the height and position (inside or outside) of the leaf within the canopy (Cowart & Graham 1999). Consequently, to reduce this variability, one needs to be more specific about the position of leaves collected. Additionally, a larger sample size of trees than used here is recommended, as this would help detect trends within such ‘noisy’ data.

The lack of relationship between oviposition by mopane moths and FA in mopane trees is in accordance with results from a similar study on mopane by Wiggins (1997). Similarly, Bañuelos *et al*. (2004) found there to be no relationship between foliar FA in *Rhamnus alpines* and its susceptibility to insect herbivory. Due to the common finding that ovipositing females select host plants that provide the qualitatively best food for their larvae (Rausher 1981; Denno *et al.* 1990, Dini & Owen-Smith 1995; Lower *et al*. 2003), both these studies suggest that a reason for the lack of relationship could be that FA is not related to herbivore defence. This study, however, has shown that foliar FA in mopane does increase with a decrease in defensive compounds. Therefore, the lack of relationship between host choice and foliar chemical composition found previously (Chapters 5 and 6) is confirmed here. As discussed in Chapters 5 and 6, this could be due various reasons, such as the short adult life-span of the moths, which limits their temporal scope for sampling among trees; or the selection for resource quantity rather than quality. Additionally, the relationship between foliar chemistry and FA was only found at a very broad scale and hence, even if moths did try to select for more nutritious trees, FA would not be a very good indicator of this at the individual tree level.

In conclusion, leaf FA does appear to be an indicator of plant stress in mopane trees, as on a broad scale, developmental instability increased with a decrease in defensive chemical compounds in the leaves. These stress related changes in leaf chemistry were presumably due to environmental conditions rather than herbivory, however, as neither intense defoliation nor pruning had any effect on leaf FA, even though changes in leaf size and chemical composition occurred. The relative impact of each browsing type could hence not be determined by measures leaf FA in this study, but the extreme tolerance of mopane to intensive herbivory (defoliation and pruning) is made evident. Additionally, due to the poor relationship between FA and leaf chemistry, it is not surprising that oviposition by mopane moths was also not related to leaf FA. It should be noted, however, that there is a possibility that FA is not actually a sensitive indicator of plant stress (Bjorksten *et al.* 2000; Møller 2000), and the strength of this relationship therefore requires further investigation.

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CHAPTER 8

GENERAL CONCLUSIONS

Pruning by elephants and defoliation by mopane caterpillars each had a significant but different effect on the regrowth responses of mopane trees, yet neither type of herbivory had any influence on reproductive investment or leaf fluctuating asymmetry in individual trees.

Regrowth morphological characteristics, namely the increase in leaf and shoot size after pruning and the decrease in leaf and shoot size after defoliation, were as expected considering the difference in plant parts and biomass quantity removed by the two browsers, as each damage type would have had a differential effect on the resource availability and allocation within the plant. The similarity in the change in leaf chemistry (decreased tannin:protein ratio) after both natural elephant pruning and mopane caterpillar defoliation was however, surprising, as woody species often respond to insect defoliation by increasing their chemical defences. What this suggests, is that mopane has adopted a tolerance strategy to herbivory, rather than one of defence, as the reduction in chemical defences would increase the plants vulnerability to further herbivory, but would also allow for the rapid growth of new leaves that could then aid in the accumulation and replacement of lost resource stores. Additionally, despite the differences in regrowth after defoliation and pruning, both types of response would promote the replenishment of resources, and hence improve the plants’ tolerance to herbivory. For example, the extended life span and size of leaves after pruning and the increased N content of leaves after defoliation all increase a plant’s photosynthetic capacity. Furthermore, the pattern of resource allocation to below ground storage organs (i.e. the large root system) prior to damage is an indication of mopane’s tolerance ‘strategy’.

Neither defoliation nor pruning had a negative effect on the reproductive investment by mopane that same season, demonstrating once again mopane's tolerance to both browsing types. The ability to reproduce, even after severe defoliation and pruning, is an indication that reproduction in mopane is determined by the quantity of stored resources rather than the current acquisition by photosynthetic material. Confirming this, defoliation also had no impact on a tree's likelihood of flowering that season, which was instead determined by tree height (i.e. size of storage organs). Additionally, the lack of impact by herbivory was probably because the availability of resources for reproduction that season would not actually have been affected by either browsing type. Regrowth on defoliated trees took place after the onset of reproduction, meaning that resources for flowering and pod production were still unaffected, for instance. Regrowth after pruning did occur prior to reproduction, however, yet the increased root/shoot ratio after pruning would have resulted in increased resource availability per remaining branch, thereby enabling reproduction despite the prior use of stored resources for regrowth. It should be noted, however, that while this study only looked at the immediate effect of herbivory on reproduction, the need for a more long-term study has become apparent, as defoliation and pruning may instead have a delayed effect on reproduction, though their effect on regrowth responses and the resultant resource accumulation rate.

Most surprising about mopane's response to herbivory, was the lack of impact caterpillar defoliation and elephant pruning had on leaf fluctuating asymmetry, as increased and retarded leaf growth, as well as decreases in chemical defences are usually associated with developmental instability i.e. stress. Additionally, the degree of damage inflicted by each herbivore was relatively severe compared to studies on other species where increases in developmental stability were observed. Mopane's apparent high degree of tolerance to herbivory is therefore confirmed. However, the relationship between FA and leaf chemistry when trees from a number of areas were considered simultaneously (i.e. from a variety of habitats) suggests that unlike herbivory, environmental conditions could have a stressing affect on mopane trees. This is possibly because mopane tends to grow in resource limited areas, where only a small degree of variation could prove stressful. To be able to measure the comparative stressing affect of defoliation and pruning, trees growing in stressful environmental conditions should therefore be considered in future studies.

With regards to intraspecific host choice by ovipositing mopane moths, mopane tree size rather than leaf and shoot characteristics was found to be the primary determining factor of oviposition behaviour. This was even the case after elephant utilisation of mopane trees, where leaf nutritional value was significantly improved. Resource quantity (measured as canopy volume) rather than quality therefore appears to influence oviposition. However, moths only displayed a low degree of selectivity for large trees, as preference was only evident at the habitat scale (avoiding scrub areas with a low mean tree height). These findings are as expected for a species with a short adult life span and larvae with eruptive population tendencies, as females are unlikely to be able to afford the time to be too selective, and density dependence during population outbreaks would drive the need for resource quantity over quality. Furthermore, the lack of selectivity according to the more detailed tree features (leaves and shoots) is not surprising, as the degree of intraspecific variation in these traits is relatively low and hence irrelevant compared to selection across species.

Elephant pruning of mopane trees resulted in significant changes in these detailed plant features, yet still oviposition by mopane moths was not influenced by leaf and shoot characteristics. There was, however, a negative association between heavy elephant utilisation of mopane woodland and mopane caterpillar abundance, due to the negative impact elephants had on the density of tall mopane trees in these areas. These two taxonomically different species do therefore interact through their common food resource, namely the above-ground browse material of mopane trees. While this interaction is initiated through the reduction in tree size after stem/branch breakage by elephants, it is also likely to be maintained (or strengthened) in the long-term due to the repeated utilisation of elephant-damaged trees by elephants (i.e. hedging). Such hedging prevents the recruitment of taller trees, which would not only affect the suitability of an area to ovipositing mopane moths at the time, but could also influence tree density in the future by reducing the number of reproductive-sized trees. This association therefore raises implications for the conservation of mopane caterpillars, as any factor increasing the degree of elephant impact on mopane trees (e.g. fences and waterholes) will also influence mopane caterpillar abundance, as well as the subsequent biodiversity in the area.

APPENDIX A

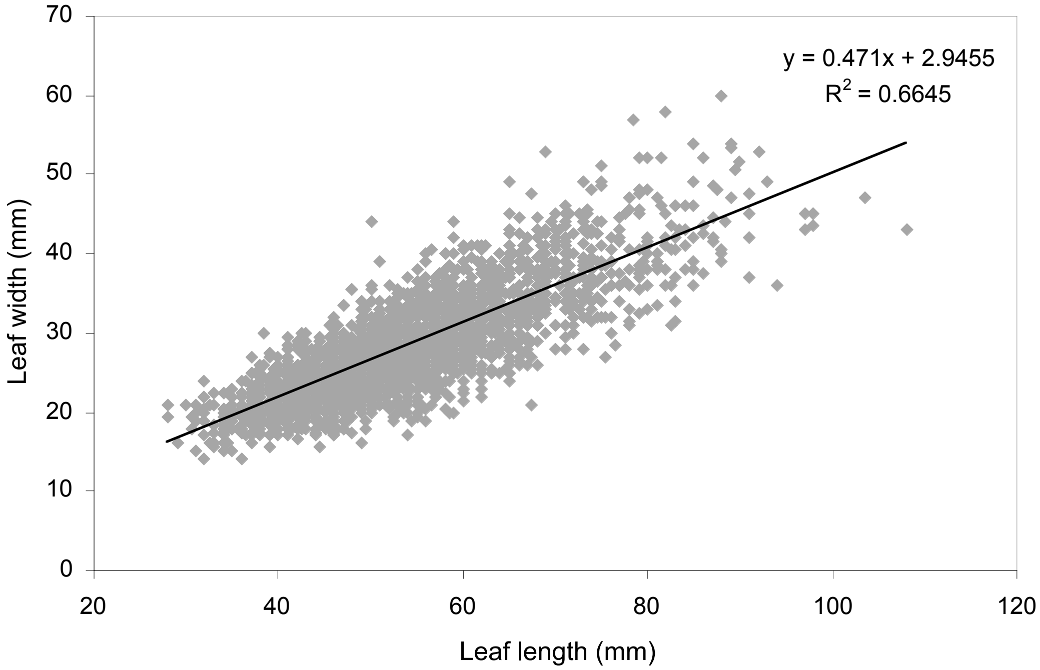

Figure A.1 The relationship between mopane leaf length and width (N = 2834).

APPENDIX B

SHOOT GROWTH

B.1 The effect of browsing treatment on shoot growth rate of mopane

At the start of the growing season, shoot length (mm) was measured for 10 shoots per tree for all trees subjected to simulated elephant and mopane caterpillar utilisation treatments (Chapter 3, 15 trees per treatment, 6 treatments). Random shoots were first measurements on the 1st of November 2003, then another set of random shoots were measured six days later, on the 7th of November. In February 2004, 5 shoots from each tree were measured, as part of the study in Chapter 3.

For the two November measurements, one-way ANOVAs were used to test for significant differences between treatments. To determine which treatments were significantly different from each other, a Tukey test was then used. Data was however, first square-root or Log transformed, as it was not normally distributed. For the February measurements, a Kruskal-Wallis ANOVA was used to test for differences between the treatments, as the data was not normally distributed, even after transformations. A multiple comparison test was then used to determine which treatments were different.

On the 1st February, when shoot growth had just began, mean shoot length did not differ between treatments (Table B.1). The time of flush does therefore not appear to be influenced by previous utilization. Six days later, however, trees pruned that dry season (in August 2003) had significantly longer shoots than trees defoliated in February 2003 (both CN&F and CF'03 trees). Because shoots were initially similar in length, it can then be concluded that the greater length of these shoots was in fact due to an increased growth rate induced by pruning. The poorer growth rate on control and defoliated trees was also not simply due to shoots having reached their maximum length in that time, as the mean shoot length was notably longer later in the season (Table B.1).

Table B.1 Mean (± SE) shoot length values measured three times during the growing season on mopane trees previously subjected to various elephant pruning and mopane caterpillar defoliation treatments. Letters indicate significant differences between treatments at each time of measurement.

Treatment[#]	Mean shoot length (mm)		
	1 November 2003	7 November 2003	11 February 2004
CF’03	18.0 ± 2.84 (a)	34.7 ± 2.67 (a)	52.3 ± 2.70 (a)
CN&F	14.7 ± 3.28 (a)	35.5 ± 3.58 (a)	55.7 ± 4.85 (a)
CN’02	13.9 ± 2.76 (a)	51.2 ± 6.30 (ab)	63.0 ± 3.46 (a)
C	11.6 ± 1.84 (a)	45.9 ± 4.64 (ab)	69.9 ± 2.21 (ab)
ET’02	16.8 ± 4.40 (a)	53.7 ± 7.48 (ab)	110 ± 10.4 (b)
ET’03	14.0 ± 3.46 (a)	61.0 ± 9.15 (bc)	158 ± 6.92 (c)

[#] Treatments included: simulated caterpillar defoliation in February 2003 (CF’03), November 2002 and February 2003 (CN&F) and November 2002 alone (CN’02), controls (Con), and simulated elephant pruning in August 2002 (E’02) and August 2003 (E’03).

B.2 Shoot growth rate over time

To investigate how the rate of shoot growth changes with the amount of time after the onset of growth, the growth of 5 shoots per tree was monitored for 15 trees that had recently been defoliated by mopane caterpillars in December 2003/January 2004. Shoots were marked at their base with colour tape and numbered accordingly. They were measured four times, on the 26th and 31st of January 2003, and on the 4th and 9th of February (i.e. at 4 or 5 day intervals). The daily growth rate (cm/day) of each shoot was then calculated according to the number of days between each measurement, and the mean growth rate was determined for each tree.

Mean shoot length at the time of the first measurement was 11.2 cm. During the first 5 days shoot growth was quite rapid for most trees, reaching up to 3.84 cm/day. After just 5 days the mean shoot length had doubled and was 22.8 cm. Growth slowed down considerably during the next four days (i.e. days 4-9), however, with the maximum growth rate decreasing to 0.65 cm/day. Between days 10 and 14, only 10% of the shoots were recorded as having grown at all and the maximum growth rate was 0.08 cm/day. Growth is therefore most rapid at the start of the flush and slows down quickly once shoots reach their final length (Fig. B.1). The short period of time mopane takes to re-flush after defoliation is also highlighted here, as shoot growth was complete within about three weeks (which includes an extra week prior to the first measurement here).

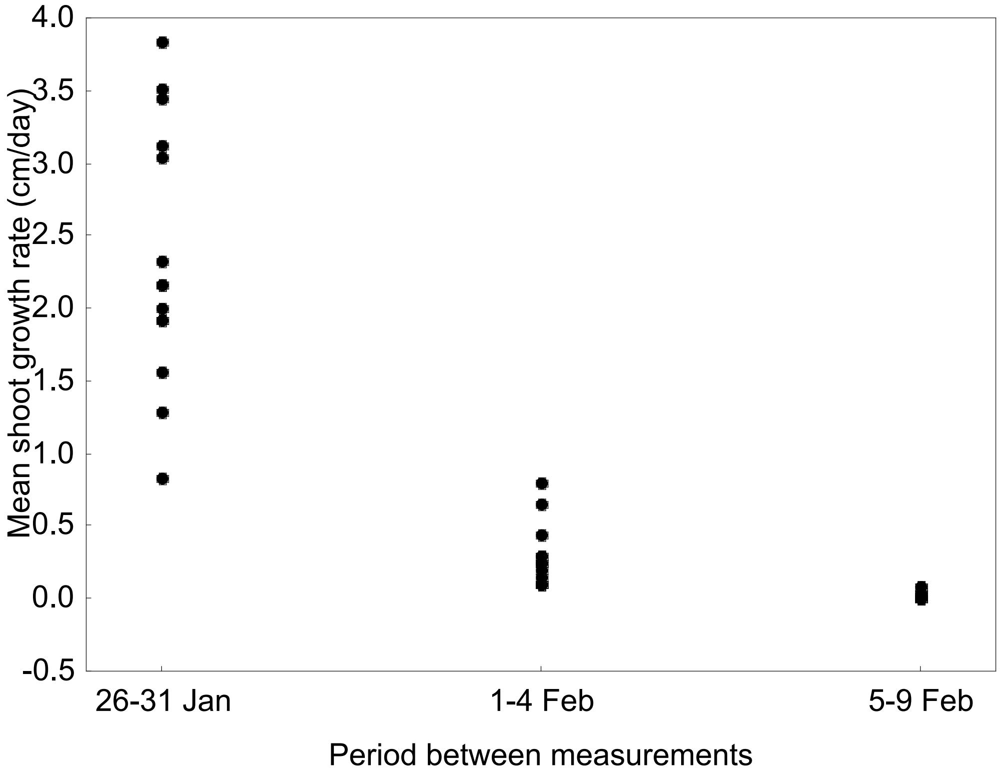

Figure B.1 The change in growth rate of mopane shoots with increasing time since defoliation by mopane caterpillars.

APPENDIX C

Table C.1 The mineral content and water pH of soil samples collected at each experimental 'tree group' along three different transects (for Chapter 3).

Transect	Tree group	Water pH	P (mg/kg)	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Total N (mg/kg)
1	1	7.2	29.2	447	258	265	77	0.01
	2	7.6	45	890	438	317	69	0.03
	3	7.4	48	609	330	293	64	0.02
	4	7.0	48	611	273	255	77	0.03
	5	7.3	30	802	389	425	68	0.02
2	6	7.2	31.8	626	298	374	62	0.02
	7	7.3	37.4	663	354	392	61	0.02
	8	7.2	58.5	687	365	354	63	0.02
	9	7.3	27.3	821	422	360	75	0.02
	10	7.2	42.6	721	327	288	68	0.02
3	11	7.5	37.2	620	368	372	67	0.02
	12	7.5	24.1	747	416	429	69	0.02
	13	7.5	28.2	912	437	476	60	0.03
	14	7.3	36.4	900	407	425	65	0.02
	15	7.4	28.2	740	342	389	65	0.03

Table C.2 The mineral content and water pH of soil samples collected along transects in the three different mopane vegetation types.

Habitat type	Transect	Water pH	P (mg/kg)	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Total N (mg/kg)
Riverine	1	6	49.2	2290	699	436	92	0.080
	2	6.1	20.1	1360	380	253	69	0.086
	3	6.2	52.4	1260	272	165	78	0.046
	4	6.4	35.2	1210	417	215	73	0.054
	5	6.9	27.5	4220	824	375	106	0.083
Woodland	1	5.7	30.4	729	345	350	86	0.026
	2	5.7	58.3	782	289	227	81	0.032
	3	5.9	51	625	289	218	86	0.029
	4	5.9	59.7	615	338	289	89	0.027
	5	6	36.9	966	417	338	88	0.031
Scrub	1	6.9	4.9	469	93	76	78	0.033
	2	6.8	4	265	124	72	80	0.028
	3	6.6	9.3	520	94	68	81	0.057
	4	6.5	3.6	973	325	342	76	0.023
	5	6.5	20	353	114	56	82	0.032

APPENDIX D

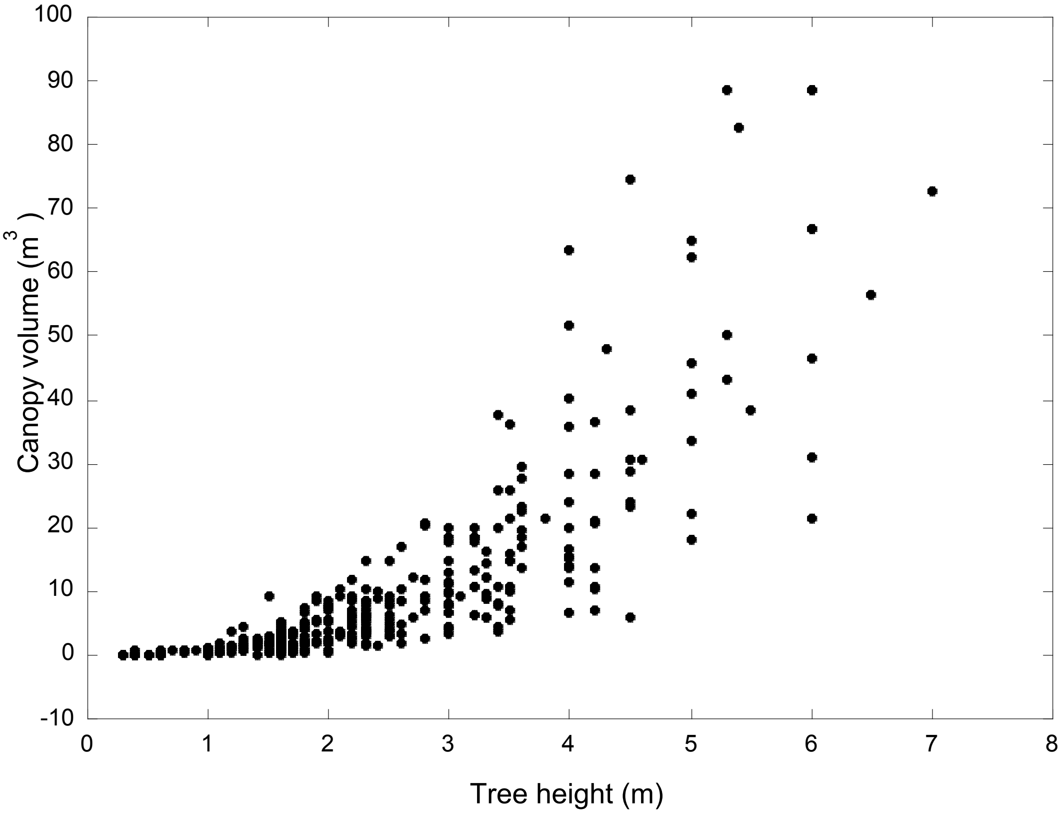

Figure D.1 Canopy volume versus tree height for all mopane trees measured in the Kruger National Park.

APPENDIX E

VOLATILE ORGANIC CARBON EMISSIONS FROM MOPANE TREES

E.1 Brief introduction and methods

Monoterpenes are biogenic volatile organic compounds (VOCs) released by plants, with the main ecological role of deterring feeding or oviposition by generalist herbivores (Pare & Tumlinson 1999; Kessler & Baldwin 2001; Pichersky & Gershenzon 2002). Interestingly, mopane trees emit monoterpenes at a rate significantly higher than most (perhaps all) other savanna tree species in southern Africa (Otter *et al*. 2002), which may explain why for invertebrates, mainly specialist species utilise mopane (e.g. mopane caterpillars and mopane psyllid).

Studies have shown that the release of VOCs increases in response to herbivore attack (e.g. Turlings *et al*. 1990; Pare & Tumlinson 1997; Priemé *et al*. 2000; Vuorinen *et al*. 2004). This response can be immediate, due to the rupturing of pre-existing internal or external secretary structures in which volatiles are synthesised and stored, or slightly delayed, occurring hours or days after feeding (Pichersky & Gershenzon 2002). Work looking at the effect of herbivory on VOCs emitted from regrowth, months after damage, is however minimal. These emissions could have a significant influence on the vulnerability of trees to further herbivory, however, as they are an indication of food quality (Tscharntke *et al*. 2001).

In February 2003, the effect of prior elephant pruning and mopane caterpillar defoliation on mopane tree VOC emissions was therefore recorded from the regrowth of trees previously subjected to simulated utilisation treatments, together with unutilised trees (same trees as in Chapter 3; sample sizes were: elephant = 9, caterpillar = 11, control

= 10 trees). Emissions were measured from one leaf per tree, using a portable dynamic (open flow), leaf cuvette. For details on the methodology, refer to Otter *et al*. (2002).

The immediate affect of caterpillar damage to VOCs was also investigated, by using the same leaves from the control and caterpillar treatment trees as mentioned above. A section of each leaf was torn off by hand, to simulate caterpillar damage, and emissions were collected 3 minutes and 15 minutes after the time of damage. From this it could be determined how the rate of emissions changed with time since damage (the total number of trees for which all three measures were taken was 19). It is recognised, however, that mechanical damage can have a different effect on VOCs compared to natural herbivore attack, as caterpillars not only take longer to inflict the same degree of damage, but they can also introduce saliva-derived compounds to the wound site, which affect emissions (Kessler & Baldwin 2001; Karban & Agrawal 2002). Therefore, VOCs from leaves with and without natural mopane caterpillar damage were also measured. Here, for four trees, emissions were collected from a leaf being fed on by a mopane caterpillar (the caterpillar was first removed), as well as from a neighbouring leaf without caterpillar damage. Lastly, to investigate whether host choice by ovipositing mopane moths was related to the level of VOCs, emissions were collected from six trees with egg masses and six neighbouring trees without egg masses.

Samples were analyzed by GC-FID (SRI 8610C; USA) using Mxt 624 column (30m x 0.25µm x 1.4µm; Restek, USA) and a temperature program. The GC (gas chromatographer) is fitted with 2 pre-concentration traps so that VOCs can be determined at the low ppb range. The first trap (filled with Tenax TA; Restek, USA) is cooled to 0°C after which the sample is transferred, by heating the trap electrically to 180°C, to the second trap (containing glass beads), which is cooled in liquid nitrogen. This is then also heated in the same manor to transfer the sample, at a flow rate of 5ml/min, onto the column. Calibrations were done with a neo-hexane standard (200ppb; AirProducts, Europe) and neat GC samples (Restek, USA) for peak identification. The level of

E.2 Results

The emission rate of _-pinene was higher in both previously pruned and defoliated trees compared to control trees (although not significantly; Fig, E.1; ANOVA: $F_{2,27} = 3.31$, P = 0.06). Mopane trees therefore appear to increase the production of monoterpenes in regrowth after both types of herbivore damage. This is interesting, considering the level of tannins and total poylphenols decreases after pruning and defoliation (Chapters 3 & 6).

Unsurprisingly, emissions were significantly higher three minutes after simulated caterpillar damage than before the damage took place (Fig. E.2; Friedman ANOVA: $\square^2 = 28.21$, df = 2, $P < 0.01$). The marked increase in _-pinene soon after damage is, however, probably due to the sudden release of VOCs from damaged storage structures rather than an increase in production, as emissions decreased again after 15 minutes, when most of the compounds in ruptured cells would have been emitted. This apparent lack of increase in _-pinene production in damaged leaves is confirmed by results from naturally eaten leaves, where no difference in the level of _-pinene was detected in caterpillar-damaged and undamaged leaves (paired t-test: $t = -1.13$, df = 3, $P = 0.340$). This does, however, require further investigation as leaves from the same tree were compared, while the tree may respond as a whole i.e. neighbouring, caterpillar-free trees should perhaps have been used. Additionally, the sample size of naturally damaged leaves was very small (4 trees).

There was no significant difference in the level of _-pinene emissions from mopane trees with and without egg masses (paired t-test: $t = -0.089$, df = 4, $P = 0.934$). Ovipositing mopane moths therefore don’t appear to select host trees according to monoterpene emissions, yet this could be because the degree of variability between trees was not sufficient within the area sampled here. Instead, differences on the habitat scale may have an influence.

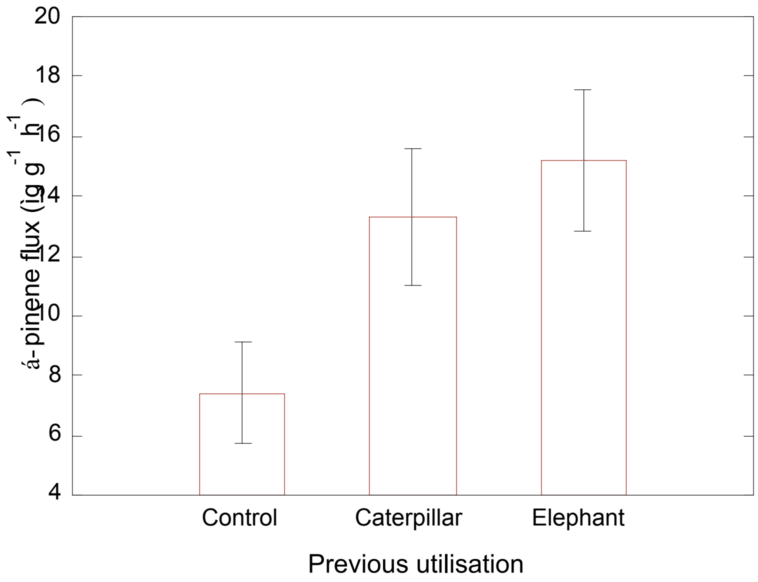

Figure E.1 A comparison of _-pinene emission rates (mean ±SE) from the regrowth of mopane trees previously subjected to simulated elephant pruning and mopane caterpillar defoliation, as well as from unutilised trees.

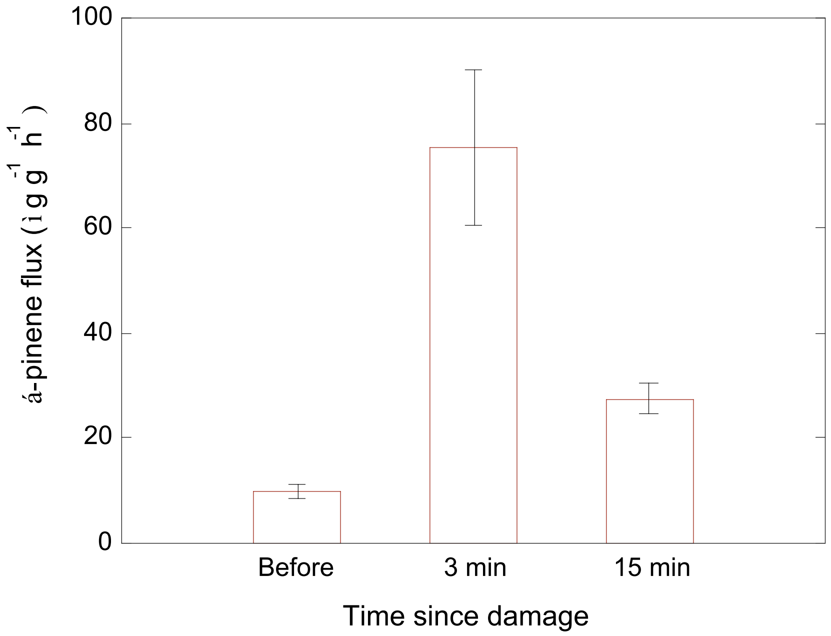

Figure E.2 The emission rate of _-pinene (mean ± SE) from mopane leaves just prior to simulated caterpillar damage and 3 and 15 minutes after damage.

E.3 References

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