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

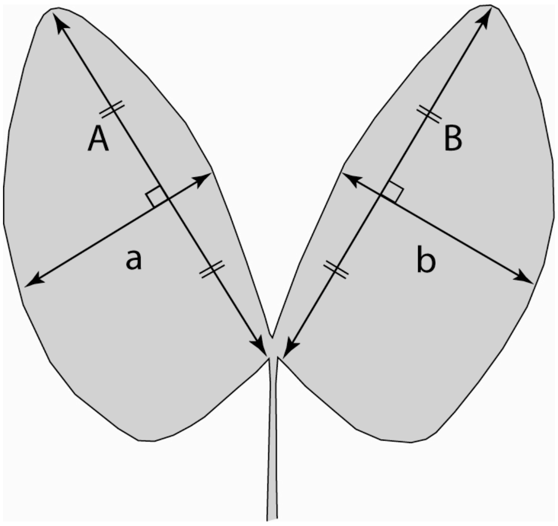

Figure 7.1 A diagram of a mopane leaf, showing the measurements taken to determine fluctuating asymmetry. Asymmetry (FA) in: length = (A-B) and width = (a-b). Absolute asymmetry_FA_in: length = _A-B_and width = _a-b_.

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: $\chi^2 = 0.168$, 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.

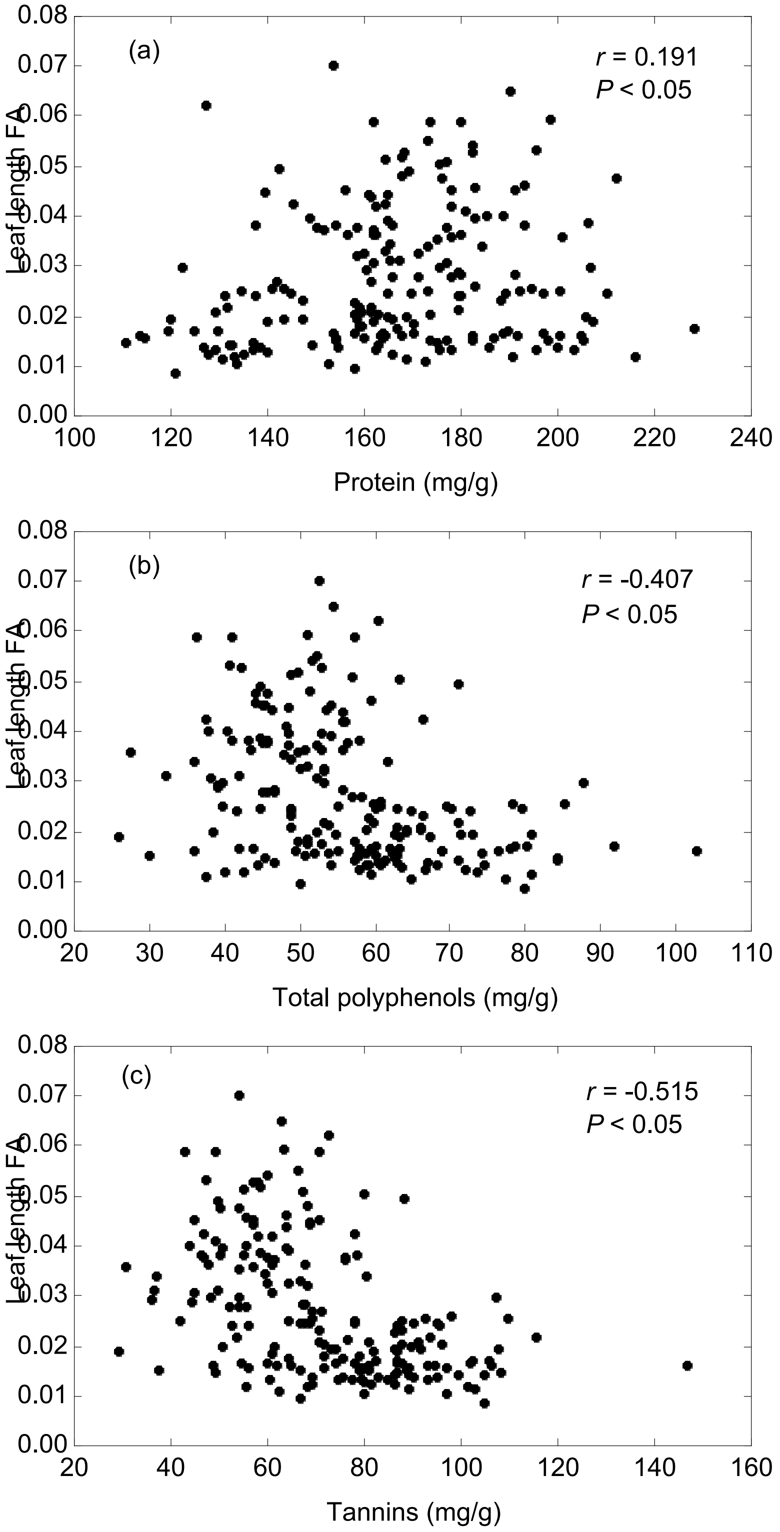

Figure 7.2 The relationship between mean fluctuating asymmetry in leaf length (_log R – log L_) and foliar (a) protein, (b) total polyphenolic and (c) tannin content in mopane trees, for all study trees combined.

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.