APPENDIX A

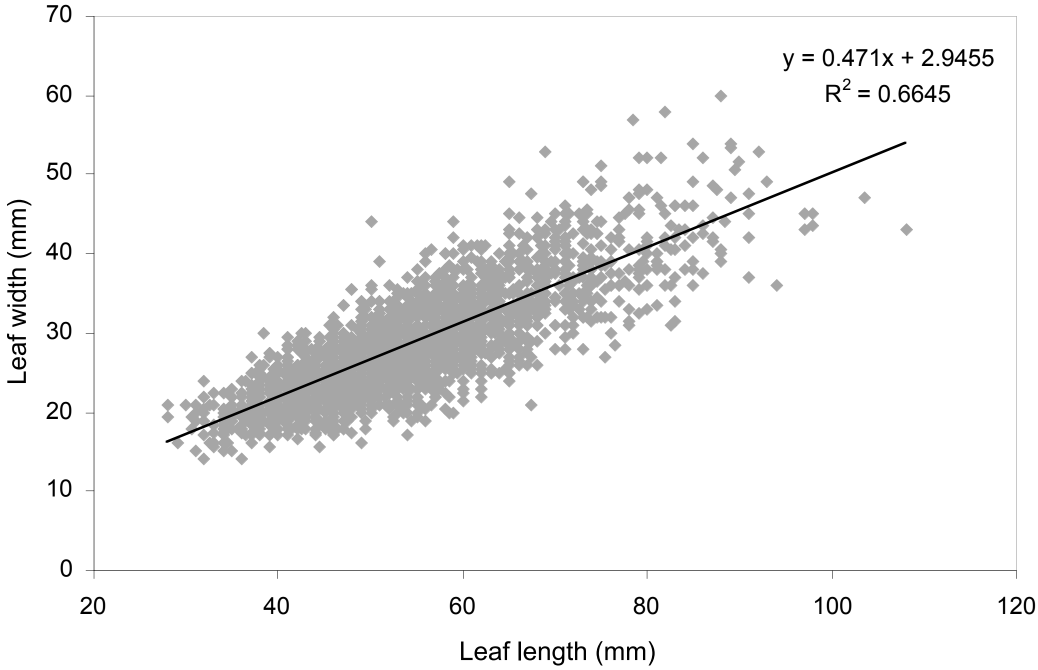

Figure A.1 The relationship between mopane leaf length and width (N = 2834).

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).

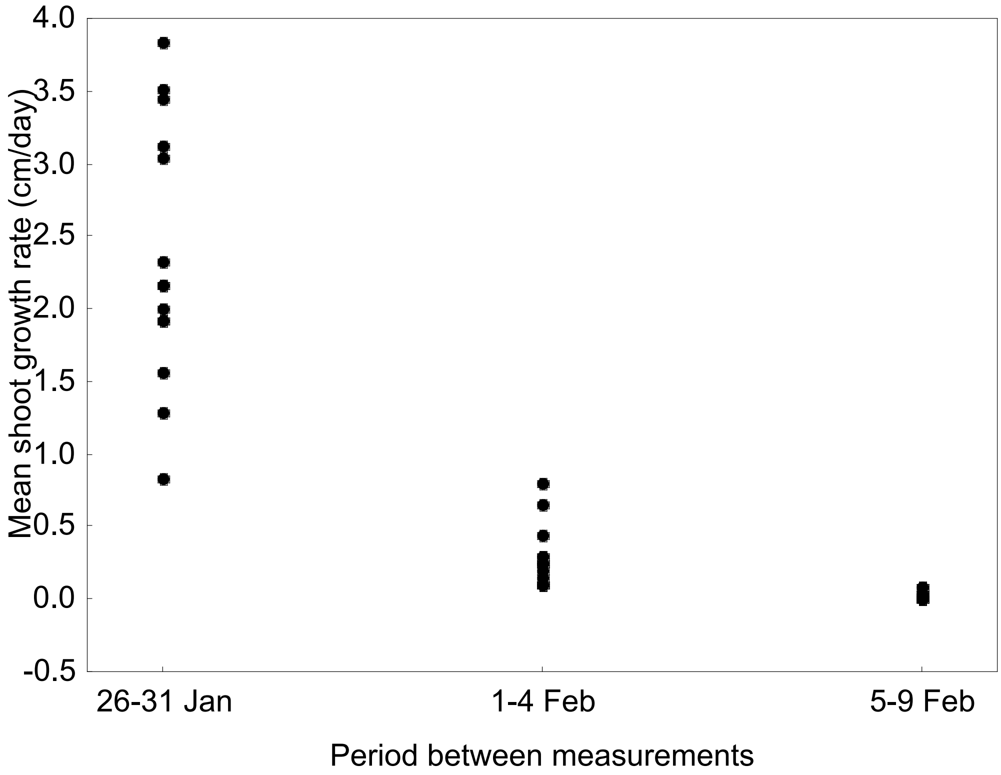

Figure B.1 The change in growth rate of mopane shoots with increasing time since defoliation by mopane caterpillars.

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

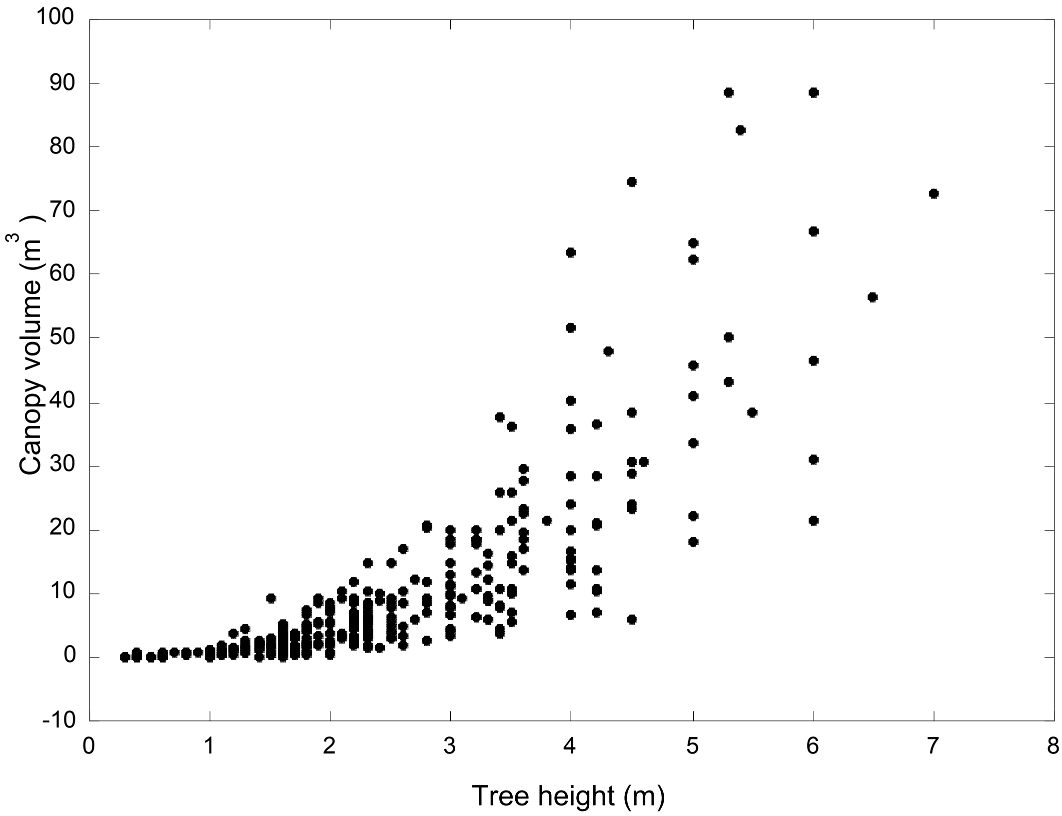

Figure D.1 Canopy volume versus tree height for all mopane trees measured in the Kruger National Park.

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.

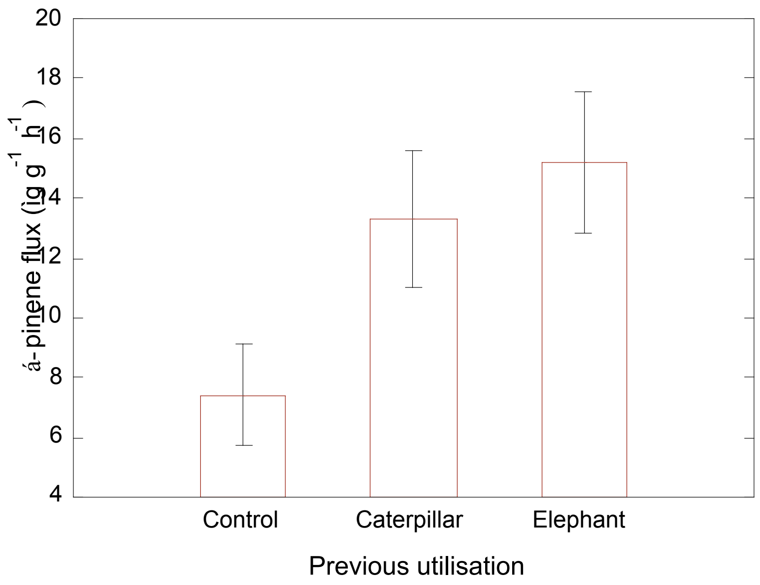

Figure E.1 A comparison of _-pinene emission rates (mean ±SE) from the regrowth of mopane trees previously subjected to simulated elephant pruning and mopane caterpillar defoliation, as well as from unutilised trees.

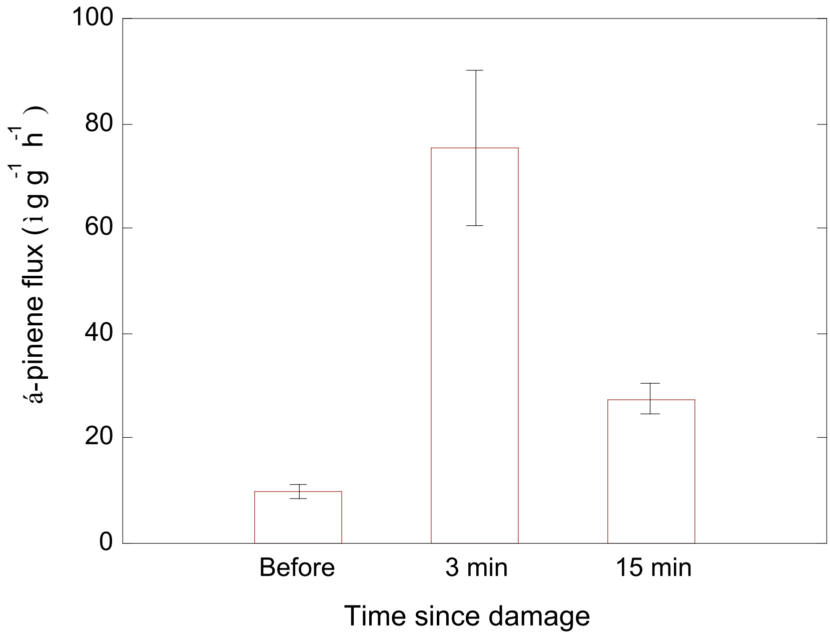

Figure E.2 The emission rate of _-pinene (mean ± SE) from mopane leaves just prior to simulated caterpillar damage and 3 and 15 minutes after damage.

E.3 References

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