

## Methods for monitoring herbivory and growth of New Zealand mistletoes (Loranthaceae)

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**Abstract:** This study provides the first quantitative comparison of methods for monitoring herbivory and growth of New Zealand beech mistletoes (*Alepis flavida*, *Peraxilla colensoi* and *Peraxilla tetrapetala*). Four monitoring methods - leaf maps, volume estimates, visual estimates of browse and foliage density, and repeat fixed-point photographs - were used to assess the health of 60 permanently tagged mistletoe plants in four South Island beech forests between February 1997 and February 1998. Leaf maps provided the most detailed information but were extremely labour-intensive so could only be used to monitor a small number of plants. Photographs were much faster to use, and the results corresponded well to leaf map data, but *A. flavida* could not be photographed because it was frequently hidden by host foliage. Visual scoring methods and volume measurements did not correlate well with leaf maps, probably because leaf loss and growth were not obvious without images of plants from previous seasons. Thus, photographs can provide valuable reference points for future evaluation of plant condition. However, because photos require more time and money than visual scoring and can only be used on a subset of the population, their most practical use is as a supplement to visual scoring.

**Keywords:** *Alepis*; herbivory; Loranthaceae; mistletoe; monitoring; *Peraxilla*; possum; *Trichosurus vulpecula*.

## Introduction

Biodiversity monitoring is "the systematic measurement of variables and processes over time in order to ascertain the degree of deviation from some expected condition" (Hellawell, 1991). Increasing interest in biodiversity monitoring has led to the development of numerous monitoring programmes in New Zealand (Norton, 1996), many of which focus on the effects of vertebrates on native plant species. No browsing or grazing mammals occupied New Zealand prior to European settlement, and although moas once grazed lower vegetation, New Zealand had no native arboreal browsers. Thus, introduced mammals such as the Australian brushtail possum (*Trichosurus vulpecula*) have been blamed for the decline of many native plant species (Brockie, 1992).

One method of estimating the importance of vertebrate herbivory in a habitat is to monitor individuals of plant species that are particularly susceptible to herbivory. Meads (1976) conducted the first such monitoring programme in New Zealand between 1969-1974 on the effects of possums on the vegetative growth of northern rata (*Metrosideros robusta*). Other studies since then have been conducted on various plant species

(Payton, 1983, 1988; Leutert, 1988; Cowan *et al.*, 1997; Payton *et al.*, 1997; Pekelharing *et al.*, 1998a, b). Native mistletoes (Loranthaceae) have been used as an indicator of the success of possum control operations in improving vegetation condition in beech forest (Rance and Rance, 1995; Sessions *et al.*, 2001), as mistletoes are considered highly palatable (and vulnerable) to possums (Wilson, 1984; Owen, 1993).

Wilson (1984) was the first to monitor three native mistletoe species (*Alepis flavida*, *Peraxilla colensoi* and *Peraxilla tetrapetala*), in a study conducted between 1978 and 1984 in Nelson Lakes National Park. Wilson recorded the size and apparent damage on 46 mistletoes and found that over the 6-year period, 41 % of the plants died (P.R. Wilson, Landcare Research, Nelson, N.Z., *unpubl.*). *Peraxilla* plants were browsed once per year on average and were more than 50% defoliated in over half of those attacks (Wilson, 1984). In the only other quantitative study of mistletoe leaf loss, Owen (1993) mapped leaves on 40 *Peraxilla colensoi* in the upper Haast Valley and found that possums had no significant effect. More recent mistletoe studies have relied on visual estimates of plant condition or browse levels, and these studies have also presented varying results [see Sessions (1999) for a review]. However, many of

these studies have used different scales to categorise browse and foliage density (see Milne, 1996), which has made it difficult to compare studies from different sites and times.

This study provides the first quantitative comparison of the monitoring methods commonly used for New Zealand mistletoes: leaf maps, volume estimates, visual estimates of browse and foliage density, and repeat fixed-point photographs. These methods were used to assess the health of 60 tagged mistletoe plants in four South Island beech forests between February 1997 and February 1998. The goal was to identify the most accurate and practical method for assessing possum damage and resulting declines in mistletoe plant and population health.

## Methods

### Monitoring sites

Six mistletoe populations were selected at four South Island beech forest sites: Craigieburn (43°09'S, 171°43'E), Lake Ohau (44°12'S, 169°49'E), the Eglinton Valley (44°58'S, 168°01'E), and Waipori Gorge (45°55'S, 170°2'E). Silver beech (*Nothofagus menziesii*) forest dominates Waipori, which is the lowest site (40 m a.s.l.) with the lowest annual rainfall (500-1200 mm). Silver beech, red beech (*Nothofagus fusca*) and mountain beech (*Nothofagus solandri* var. *cliffortioides*) forest covers the Eglinton Valley, which is at 350-380 m a.s.l. with an annual rainfall of 2300 mm. Both Ohau and Craigieburn are high altitude sites (540 m and 940 m respectively) with moderate to high rainfall (1200-4800 mm and 1400-2000 mm respectively) and predominantly mountain beech forest. Ten plants were permanently marked in each of the six populations: *Alepisflavida* at Craigieburn and Eglinton, *Peraxilla tetrapetala* at Craigieburn and Ohau, and *Peraxilla colensoi* at Eglinton and Waipori.

### Leaf maps

Using the methodology of Owen (1993), the leaves on ten 15-25 cm branches per plant were mapped in February 1997, and these leaves were each individually monitored for new damage at 3-month intervals (May, August, November 1997, and February 1998). The ten branches per plant typically carried about 400 leaves, and in total we mapped 25 685 leaves. On each visit, additional possum and insect damage on each leaf relative to its condition at the previous census was estimated using a 6-point scale: 0 = no damage, 1 = 1-25% gone, 2 = 26-50% gone, 3 = 51-75% gone, 4 = 76-99% gone, and 5 = 100% gone (categorised as 'abscised'). When averaging damage, the midpoint of

each of these percentage categories was used (12.5% for category 1, etc.). Damage in the form of torn and jagged leaf stubs was attributed to possums, while holes' and wavy, clean-edged patterns were categorised as Lepidoptera damage (Owen, 1993). Stick insect damage, characterised by straight, finely-milled edges (Meads, 1976), was also recorded but was uncommon in this study.

Percent loss of leaf area per plant for each 3-month season was calculated as the total leaf area lost on leaves that were present at the start of that season, divided by the total leaf area present at the start. New leaves that emerged during each season were not counted as 'at risk' or included in damage estimates during that season, because they were only present for part of that time. New leaves were included in the leaf maps from the start of the first season at which they were fully expanded. This meant that the number of leaves present at the start of each season varied as a result of losses and additions. To calculate annual rates of leaf area loss, allowing for the varying initial number of leaves each season, we compounded the four seasonal rates of loss. Hence, % annual loss  $L_A = (1 - [(1-L_1) \times (1-L_2) \times (1-L_3) \times (1-L_4)]) \times 100$  where  $L_x =$  fraction of leaf area lost on leaves present for the entire season  $x$ . This equation effectively takes the leaf survival for each season then multiplies those figures to get the compound survival. Annual leaf flux did not have to include any seasonal component that would be affected by changing numbers of leaves from season to season, so % annual flux per plant = (total number of new leaves - total number of leaves lost)  $\times$  100/(initial number of leaves), where 'number of leaves' means the sum of leaf area lost or present in leaf units. An additional set of calculations of leaf loss and leaf flux over the whole year was based on the above but also incorporated branch loss, whereby total % leaf loss = (% leaf loss)  $\times$  (% branches still alive in February 1998) - (% branches lost during the year), and similarly for leaf flux. These equations set leaf loss equal to all leaves on lost branches, plus the measured percent loss on surviving branches.

### Plant volumes and visual estimates

Every three months, plant volumes were also calculated (length north-south  $\times$  length east-west  $\times$  height), and visual estimates were made of percent foliage browsed and percent foliage density on each plant. The browse score was estimated as the percent of plant foliage removed by either insects or possums during the last six months (i.e., recognised as recent browse). Foliage density was estimated by comparing plants against computer generated images of silhouettes in 10% density classes [see Milne (1996) for a review of indices used previously to assess possum browse and foliage density of mistletoes].

## Photographs

Plants of *Peraxilla* spp. were also photographed from fixed points at 3-month intervals using 100 ASA slide film and a powerful flash (guide number 45, ASA 100, metres). A small wipeboard and ruler attached to a plastic extendable pole was placed next to the plant in each photo to provide scale, an identification number and date. Photos were always taken from photopoints defined by the compass bearing and distance (1.5-4.5 m) from the host trunk. Several photopoints had to be shifted to accommodate new growth on the plant or because of fallen debris or the angle of the sun. Few *Alepis flavida* plants were photographed because *Alepis* typically grows on the outer branches (Norton *et al.*, 1997) where it is partially hidden by host foliage. The slides recording each plant in February 1997 and February 1998 were compared by projecting the two images side by side. The percentage change in total foliage cover on each plant was estimated visually.

## Data analysis

The estimates of change over time were compared among the six populations using ANOVA tests. Leaf loss, change in volume, change in browse score, and change in foliage score were all normalised using a log transformation. Pearson's rank correlation coefficients were used to compare the estimates of change obtained from different monitoring methods. We assumed that the most numerically detailed and objective method

(leaf maps) was the most accurate and we compared other methods against it [see also Owen (1993)]. Volume, percent browse, and percent foliage density were expressed as ratios (final/initial values) and normalised with a log transformation. These changes were compared with annual leaf loss (log transformed) and leaf flux calculated from leaf maps (both including and excluding branch loss) over all six populations. The changes determined from photographs were compared with leaf loss and leaf flux from the four *Peraxilla* populations using both Pearson's and Spearman's rank correlation coefficients. Pearson's rank correlation coefficients were also calculated for plants within each population. The two plants that died during the study (one *Alepis flavida* at Eglinton and one *Peraxilla colensoi* from Waipori) were excluded from analysis.

## Results

### Leaf maps

According to leaf maps, overall leaf loss and leaf flux (including and excluding branch loss) were significantly different among the six populations (Table I). Leaf area loss was very high (96%) for *Alepis flavida* at Eglinton, moderate (40-60%) for *Alepis flavida* at Craigieburn and *Peraxilla colensoi* at Eglinton, and low (< 25%) for the remaining three populations (Table I). Both *Alepis flavida* populations and *Peraxilla colensoi* at Eglinton had negative leaf fluxes (more leaf loss than leaf

**Table 1.** Mean changes ( $\pm$  SE) in plant condition from February 1997 to February 1998 for six populations of New Zealand mistletoes ( $n = 10$  per site) according to four monitoring methods (leaf maps, repeat photos, volume measurements and visual condition scores). Annual rates of leaf loss, leaf flux and photo changes are expressed as percent changes. Changes in volume, browse, and foliage scores are expressed as ratios (final/initial values). F-values are given for differences among populations (rows) in each of the column variables.

Population	Leaf area loss (%)	Leaf flux (%)	Flux plus branch loss (%)	Change in photos (%)	Change volume	Change browse score	Change foliage score
<i>Peraxilla tetrapetala</i> Ohau	12.77	35.00	-0.78	-8.50	1.04	1.38	1.01
<i>Peraxilla tetrapetala</i> Craigieburn	10.58	53.25	53.25	2.00	1.15	0.89	1.00
<i>Alepis flavida</i> Craigieburn	36.86	-10.49	-10.49	NA <sup>1</sup>	0.91	0.86	1.51
<i>Alepis flavida</i> Eglinton	96.07	-54.07	-59.15	NA <sup>1</sup>	0.81	2.34	0.58
<i>Peraxilla colensoi</i> Eglinton	50.45	-2.83	-2.83	1.50	1.21	0.62	1.59
<i>Peraxilla colensoi</i> Waipori	21.25	29.59	27.95	0	0.74	0.68	1.31
F-value (P)	32.98 (<0.001)	14.39 (<0.001)	13.70 (<0.001)	3.48 (0.0259)	2.01 (0.0931)	3.69 (0.0062)	8.66 (<0.001)

<sup>1</sup> *Alepis flavida* populations were not photographed, as host foliage was difficult to distinguish from mistletoe foliage.

production), while both *Per axilla tetrapetala* populations and *Per axilla colensoi* at Waipori experienced net gains in leaf unit area. Branch loss contributed to leaf loss most in *Peraxilla tetrapetala* at Ohau, changing mean flux from 35% growth to a slight loss in leaf area. Branch loss also contributed slightly to leaf loss in *Alepis flavida* at Eglinton and *Peraxilla colensoi* at Waipori (Table 1).

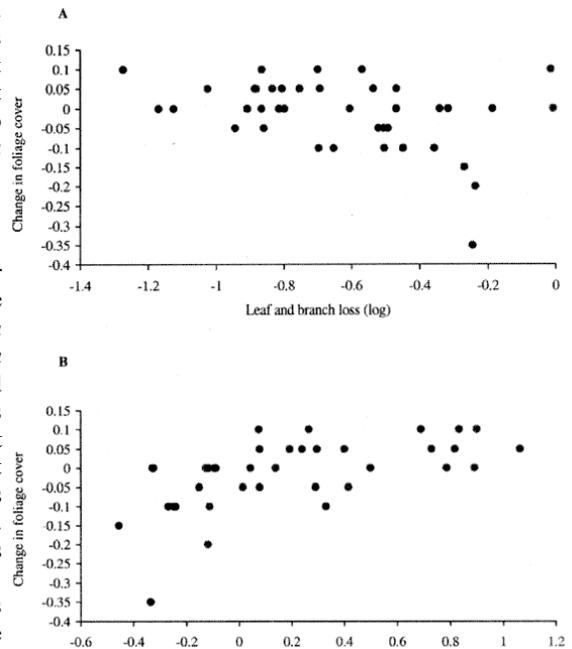
### Photographs

Photographs showed a small loss of total foliage for *Peraxilla tetrapetala* at Ohau, small increases in foliage for *Peraxilla tetrapetala* at Craigieburn and *Peraxilla colensoi* at Eglinton, and no change for *Peraxilla colensoi* at Waipori. The four *Peraxilla* populations had significantly different changes in photograph scores (Table 1). Change estimated from photographs was not significantly related to leaf loss or leaf flux without branch loss, but it was significantly related to leaf loss and flux once branch losses were included (Table 2; Fig. 1). Branch loss was readily apparent from photographs (Fig. 2).

Although these relationships had low  $R^2$  values (0.14 and 0.33 respectively), these correlations were stronger than the other relationships measured. Spearman's rank correlation coefficients also showed a significant relationship between the change estimated from photographs and both leaf loss including branch loss ( $r = -0.3675$ ,  $P = 0.0219$ ) and leaf flux including branch loss ( $r = 0.6245$ ,  $P < 0.001$ ). For the four *Peraxilla* populations, change in photos was not significantly related to annual change in percent browse ( $r = -0.295$ ,  $P = 0.0688$ ), foliage density ( $r = 0.203$ ,  $P = 0.2145$ ), or plant volume ( $r = 0.094$ ,  $P = 0.5674$ ). Although *Alepis flavida* was not systematically photographed and percent change was not calculated, defoliation was apparent on photographs of plants browsed by possums.

**Table 2.** Pearson's rank correlation coefficients between the log of changes in volume, percent browse, or percent foliage density and leaf loss (log) or leaf flux both with and without branch loss for six populations of New Zealand mistletoes ( $n = 39$  for photos, for other changes  $n = 58$ ) between February 1997 and February 1998.

	Change photos	Change volume (log)	Change % browse (log)	Change % foliage
Leaf loss	$r=0.115$ $P=0.4845$	$r=-0.107$ $P=0.4237$	$r=0.128$ $P=0.3396$	$r=-0.052$ $P=0.7003$
Leaf and Branch loss	$r=-0.376$ $P=0.0184$	$r=-0.099$ $P=0.4597$	$r=0.204$ $P=0.1238$	$r=-0.132$ $P=0.3239$
Leaf flux	$r=0.232$ $P=0.1560$	$r=0.174$ $P=0.1754$	$r=0.145$ $P=0.2761$	$r=-0.144$ $P=0.2800$
Leaf flux and branch loss	$r=0.577$ $P=0.0001$	$r=0.178$ $P=0.1423$	$r=-0.202$ $P=0.1281$	$r=0.191$ $P=0.1500$

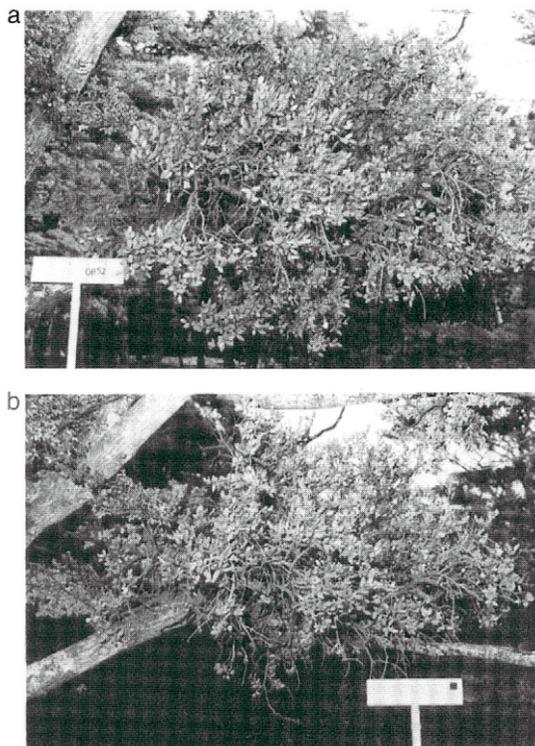


**Figure 1.** Correlations between change in foliage cover in photographs and (A) leaf loss including branch loss, where high values equal large leaf losses ( $r = -0.376$ ,  $n = 39$ ,  $P = 0.0184$ ) and (B) leaf flux including branch loss, where high values equal positive net leaf flux ( $r = 0.577$ ,  $n = 39$ ,  $P = 0.0001$ ) in four populations of *Peraxilla* spp. from February 1997 to February 1998.

### Volume, browse and foliage density estimates

Change in volume was not significantly different among the six populations, but change in both browse score and foliage score did significantly differ among the mistletoe samples (Table 1). Both *Peraxilla tetrapetala* populations increased in volume over the year, reflecting their positive leaf fluxes (Table 1). However, the browse scores for *Peraxilla tetrapetala* also increased even though little possum browse was recorded on these populations. Little change in foliage density was observed for either of the *Peraxilla tetrapetala* populations. Both *Alepis flavida* populations decreased in mean plant volume, again reflecting negative leaf fluxes. Browse and foliage density scores showed a decline in *Alepis flavida* at Eglinton but suggested an increase in condition for *Alepis flavida* at Craigieburn.

Volume, browse scores and foliage density scores all indicated that *Peraxilla colensoi* at Eglinton improved over the year, but this population had a negative leaf flux. In contrast, *Peraxilla colensoi* at Waipori decreased in size and showed no change in browse, but increased in foliage density and had a positive leaf flux (Table 1).



**Figure 2.** Photographs of a *Peraxilla tetrapetala* plant at Lake Ohau in (a) February 1997 and (b) February 1998, before and after losing its bottom branches to wind damage. Annual leaf flux for this plant was +33% when lost branches were not considered but -33% when lost branches were included.

Overall, change in mean volume corresponded to leaf flux only for populations with large positive or negative leaf fluxes but not for populations with smaller changes. Mean browse and foliage density scores did not correspond to mean leaf flux in most populations.

No significant relationship existed between any of these three estimates and either leaf loss or leaf flux with or without branch loss over all six populations (Table 2). Similarly, within each population, changes in volume, browse score and foliage density did not correspond to leaf loss or flux including branch loss except in one case, that of *Alepis flavida* at Craigieburn where browse scores corresponded to leaf loss ( $r = 0.663$ ,  $P = 0.0365$ ).

## Discussion

### Comparing monitoring methodologies

Leaf maps provided the most detailed data about leaf loss and the effects of herbivory on the study plants, as

many individual leaves were tracked on each plant through time. This method was the most objective and replicable across sites and through time because no subjective assessments of foliage cover or browse were required. Instead, the percentage of each leaf removed by insects or possums was estimated and summed for an overall estimate of plant leaf loss. However, leaf maps probably underestimated possum damage, because browsed leaves were more likely to abscise and this secondary possum damage was not recorded.

A correlation between higher than average possum damage and high abscission rates must therefore be used to infer when possums are having a large effect on plants. However, this seems to be a problem only at high levels of possum damage. Despite these problems, we consider leaf maps to be the most objective method used, as the only decision that had to be made by the person monitoring the plants was how much of each individual leaf was missing, thus minimising measurement error. We therefore used the leaf map method to assess the other methods.

It should be noted, however, that leaf maps are not a practical method of monitoring from a management perspective as they are extremely labour-intensive. Leaf maps on each plant took approximately two hours to draw initially and one hour to recheck in the field, plus substantial time later for data entry and analysis. Furthermore, only a small proportion of mistletoe plants in a population (and, in turn, only small parts of the chosen plants) can be monitored. These small sample sizes are problematic because possum browse is extremely patchy both within and between plants (Sessions and Kelly, 2001), so the amount of damage on mapped branches may not accurately reflect the overall damage on a plant or population.

Change in foliage based on photographs was the only method that corresponded to the results from leaf maps. Changes in volume, browse scores, and foliage density scores did not correspond to photograph changes. This may partially be because photographs can be used to easily identify branch loss. Branch loss was relatively uncommon (only 6.8% of branches in this study were lost), but when it did occur (e.g., in *Peraxilla tetrapetala* at Ohau), it contributed to a large amount of leaf loss. Photographs can also be useful for detecting possum browse, particularly because possums often damaged discrete sections of plants rather than spreading damage across many branches.

Unlike photographs, visual scoring methods and volume measurements did not correspond to leaf loss found using leaf maps. The difference in leaf loss estimated by photographs and visual scoring may seem surprising since the same procedure of scoring foliage cover was used in both methods. However, the important difference between the methods was that before and after photographs of a plant could be compared side by

side when determining the score. This process enabled us to note changes in individual plants through time (e.g., broken branches) as well as differences between sites and species. In addition, photos can be archived and used to assess long-term changes in much more detail than is possible with visual scores made in the field. Potentially, another problem with visual scoring in the field is differences between individuals in scoring plants, but Payton *et al.* (1997) found that two observers could obtain mean foliage cover scores of within 2.5% of each other with 95% confidence. We used foliage density scores in this study because density rather than cover had been used in past monitoring studies of mistletoes (see Milne, 1996). However, Payton *et al.* (1997) suggest that use of Foliage Cover Index (FCI) scores rather than foliage density scores could increase reliability, and this change in method could potentially increase the fit between visual scores and leaf loss on leaf maps.

In our study the same person monitored all plants to remove observer variability. Despite this, the visual scoring method was still subject to inconsistencies because the observer's frame of reference (i.e., the overall appearance of plants in the area) changed across sites and through time. For example, when most of the plants at a site increased in foliage cover, plants tended to be given relatively high scores, regardless of whether each individual plant had actually increased in foliage density. In contrast, photographs made it possible to avoid errors in visual interpretation by comparing plants through time, rather than comparing plants within a site at any given time. Volume measurements were difficult to replicate when done in the field because plants were irregularly shaped and because decisions had to be made about whether to include certain parts of plants (e.g., protruding branches, defoliated branches or dead foliage).

Photomonitoring does have some disadvantages compared with visual scoring methods. First, the causes of leaf loss are difficult to determine from photos alone, and thus a record of the cause of leaf loss (e.g., a percent browse score) must also be made in the field. Second, plants that are located high in host trees or that are intertwined with host foliage (like many *Alepis flavida*) may be difficult to monitor. This means only a subset of the population can be assessed with photographs, although many more plants (and more of each plant) can be monitored than with leaf maps. Third, photomonitoring is more expensive and more time-consuming to set up than a simple scoring system. Photomonitoring may take 10 minutes per plant to set up, whereas scoring only takes a few minutes per plant. However, once photopoints are established, photographs only take a few minutes per plant and comparisons between plants are also simple and fast. Finally, photomonitoring cannot be done successfully in all

light and weather conditions. Visual scores can be made in inclement weather, while the best light conditions for photos occur at dawn and dusk or during an overcast (but not rainy) day. These practical considerations may deter managers who have limited resources and time to commit to vegetation monitoring. Nonetheless, photographs can be valuable over multiyear periods even if the weather is not suitable for retaking them in every year.

### Recommendations for monitoring

The most important consideration for conservation managers should be to ensure that large sample sizes are monitored, because, in anyone year, possums appear to browse only a small subset of mistletoe individuals within a population. Moreover, plants should be monitored for long time periods since defoliation on individuals may be apparent but population-level decline may be more difficult to detect. Even a small increase in mortality could cause population decline in plants such as mistletoes that have long life spans and low reproductive rates.

In addition, monitoring should be conducted at relatively frequent intervals because possums may damage plants suddenly and severely within a short time. Also, browse more than six months old can be difficult to identify because damaged leaves frequently abscise (Sessions and Kelly, 2001) and new flushes of growth may replace lost leaves. For example, despite severe defoliation by possums between February and May 1997, little damage was apparent on *Alepis flavida* at Eglinton by February 1998.

Plant appearance and the relative palatability of plants to possums may also change seasonally (Ogle and Wilson, 1985; Owen and Norton, 1995). Such changes influence monitoring effectiveness. During summer, for example, fruiting and flowering can be recorded successfully (Milne, 1996), whereas during spring, flushes of new growth can lead to overestimates of plant health (Norton, 1997). Thus, ideally plants should be monitored twice each year. Plants should be photographed and given a visual score during the winter when plants have the least foliage, and a quick revisit can be conducted during the summer to evaluate visual scores in the field again and to record fruiting and flowering if desired.

### Conclusions

This study indicated that estimates from repeat photographs corresponded well to overall leaf loss and leaf flux on plants according to leaf maps. It also indicated that visual scoring methods did not correspond to leaf maps. The change in plant foliage through time

was estimated more easily and accurately by comparing photos than by scoring plants in the field without any image of the previous appearance of plants. However, although photographs provided a good estimate of overall changes in plant condition, including branch loss, they would be more expensive and time-consuming than visual scoring. Thus, the most accurate and practical mistletoe monitoring programme would be to supplement visual scoring methods (such as the Foliar Cover Index described in Payton *et al.*, 1997) conducted twice each year with photomonitoring of a subset of the population each winter. The photographs can act as benchmarks to compare plant condition across years. The overall importance of possum browse to plant health can then be assessed by examining the relationship between photographs and visual scores.

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