

Development of non-destructive age indices for three New Zealand loranthaceous mistletoes

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Abstract We investigated a variety of non-destructive measures as potential predictors of mistletoe age as determined anatomically for three mistletoe species, *Alepis flavida*, *Ileostylus micranthus*, and *Tupeia antarctica*. We show that the diameter of the host stem immediately below the haustorial attachment is consistently the best predictor of mistletoe age with R^2 values of 0.622–0.849. We suggest that host branch diameter can be used in future studies of mistletoe population dynamics and other age-dependent aspects of mistletoe ecology without destructively sampling mistletoe populations as it provides a good indication of mistletoe age for the mistletoe-host pairs we studied. However, these relationships have been derived from single sites and hosts for each mistletoe species, suggesting caution when applying them at other sites or to other hosts.

Keywords *Alepis flavida*; *Ileostylus micranthus*; *Tupeia antarctica*; Loranthaceae; mistletoe; ageing

INTRODUCTION

While it is possible to obtain accurate information on the age of many woody plants by counting the number of annual rings (Norton & Ogden 1987), estimation of age using this technique is difficult with mistletoes (Loranthaceae and Viscaceae). This may be so because the mistletoe or its host does not produce annual growth rings (e.g., Patel 1991; Reid et al. 1995) or for conservation reasons it is not considered desirable to destructively sample the mistletoe in order to age the haustorial connection (Dawson et al. 1990a). Accurate information on plant age is essential if we are to understand better the population dynamics of mistletoes. Furthermore, it has been suggested that many aspects of mistletoe biology are age-dependent (Schulze & Ehleringer 1984; Dawson et al. 1990b; Powell & Norton 1994).

Several authors have shown that it is possible to age mistletoes anatomically by counting the number of growth rings laid down by the host plant since the haustorial connection was established (Srivastava & Esau 1961; Calvin 1967; see also Menzies 1954). However, this may not always be possible or desirable. Work by Dawson et al. (1990a) with the viscaceous mistletoe *Phoradendron juniperinum* in western North America has shown that the number of bifurcating branching events on the longest stem of the mistletoe was strongly correlated with mistletoe age based on anatomical work. For the Australian loranthaceous mistletoes *Amyema quandang* and *Lysiana exocarpi*, Reid & Lange (1988) argued that the maximum diameter of the host branch proximal to the haustorium was proportional to mistletoe age because seedlings of most mistletoes established on young host branches. However, because both their mistletoes and host species did not produce clear annual growth rings, they were not able to corroborate this. Reid et al. (1995) suggested that regular vegetative growth and branching patterns (cf. Dawson et al. 1990a) can be used as an index of mistletoe age in mistletoe species that have an annual growing season.

Evidence suggests that, at least in some parts of New Zealand, loranthaceous mistletoes (*Alepis*

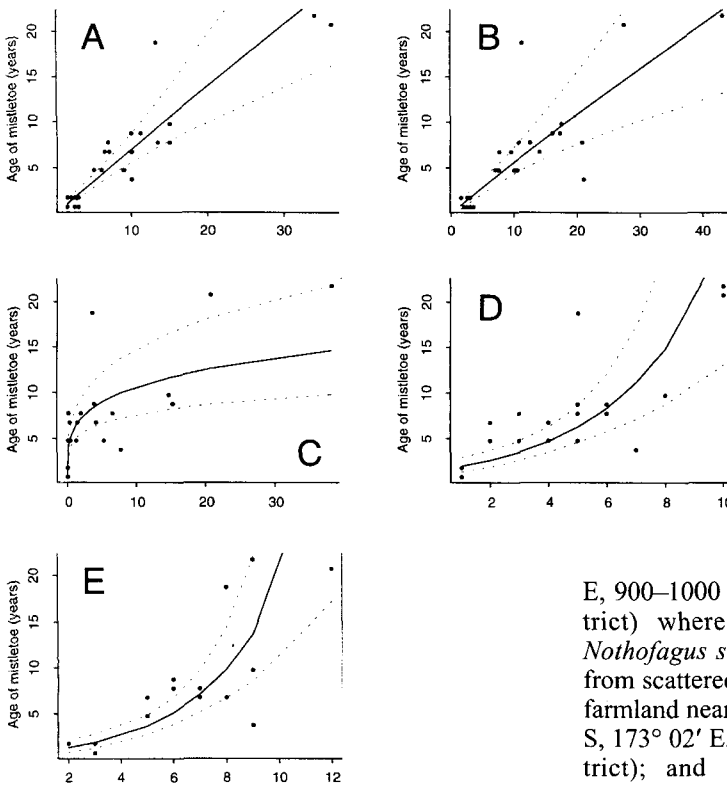


Fig. 1 The relationship between mistletoe age as determined by anatomical analysis and a number of non-destructive potential indices of age for *Alepis flavida*. The solid line is the fitted regression, with 95% confidence intervals indicated by the dotted lines ($n = 26$). **A**, Host branch diameter (mm); **B**, Mistletoe basal diameter (mm); **C**, Mistletoe volume (cm^3); **D**, Resting bud scar number; **E**, Maximum number of branch orders.

flavida, *Ileostylus micranthus*, *Peraxilla colensoi*, *Peraxilla tetrapetala*, *Tupeia antarctica*) are less abundant now than they have been in the past and there is considerable concern about their future abundance (papers in de Lange & Norton 1997; Norton & Reid 1997). Reflecting these concerns, all of New Zealand's loranthaceous mistletoes have been ranked as local or threatened (Cameron et al. 1995). Because of this, destructive methods of aging mistletoes are considered unacceptable and permission for this is unlikely to be granted on lands managed by the New Zealand Department of Conservation (P. J. de Lange pers. comm.). In this study, we sought to develop non-destructive methods to age New Zealand's loranthaceous mistletoes as a basis for better understanding their population dynamics and other aspects of their ecology.

METHODS

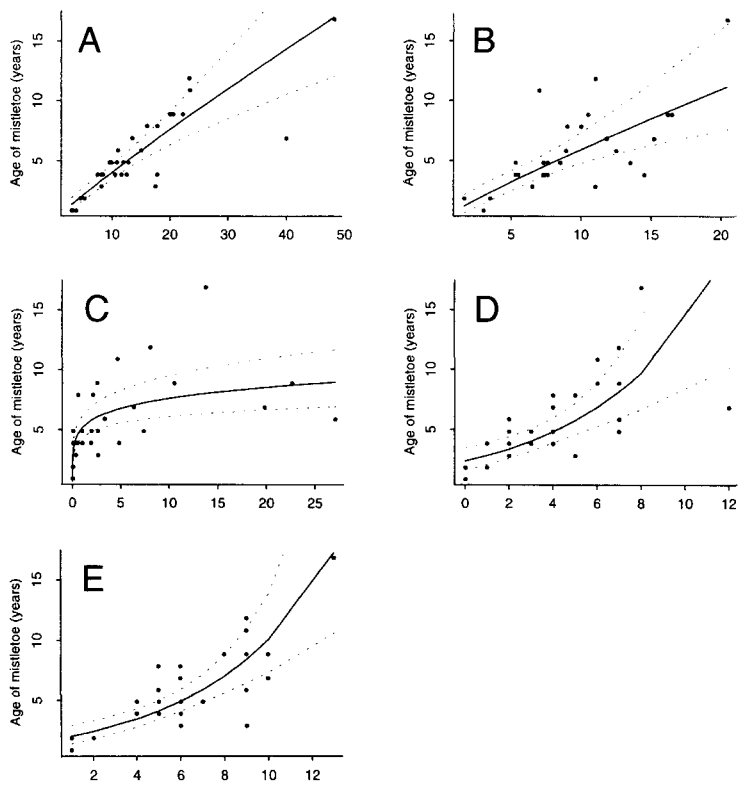
We sampled three mistletoe species at sites in northern South Island, New Zealand: *Alepis flavida* in Craigieburn Conservation Park ($43^\circ 09' \text{S}$, $171^\circ 43'$

$09' \text{E}$, 900–1000 m a.s.l., Craigieburn Ecological District) where it is common in monospecific *Nothofagus solandri* forest; *Ileostylus micranthus* from scattered remnant *Podocarpus totara* trees in farmland near Wakefield, south of Nelson ($41^\circ 25' \text{S}$, $173^\circ 02' \text{E}$, 60 m a.s.l., Moutere Ecological District); and *Tupeia antarctica* from roadside *Chamaecytisus palmensis* shrubs at Wainui on Banks Peninsula ($43^\circ 49' \text{S}$, $172^\circ 54' \text{E}$, 10 m a.s.l., Akaroa Ecological District).

At each site we aimed to sample 30 mistletoe plants spanning the full range of mistletoe plant sizes present. Where possible, we attempted to sample only one mistletoe plant per host plant to reduce impacts on hosts. However, at the Wainui site where the host plant is a naturalised shrub, we relaxed this constraint. Plants were sampled by cutting the mistletoe and its host branch off the host tree. For each mistletoe, measurements were made of the diameter of the mistletoe stem immediately above the haustorium, the diameter of the host branch immediately below the haustorium, the length of the longest axis through the mistletoe and of two axes perpendicular to this (from which we calculated the volume of the mistletoe based on the formula for an ovoid), the maximum number of annual resting bud scars present between the haustorium and branch tip on the mistletoe, and the maximum number of branch orders present on the mistletoe.

The mistletoe was then sectioned at the point of the haustorial attachment so that the age of the mistletoe could be determined anatomically based on the number of annual growth rings formed by the host

Fig. 2 The relationship between mistletoe age as determined by anatomical analysis and a number of non-destructive potential indices of age for *Ileostylus micranthus*. The solid line is the fitted regression, with 95% confidence intervals indicated by the dotted lines ($n = 30$). **A**, Host branch diameter (mm); **B**, Mistletoe basal diameter (mm); **C**, Mistletoe volume (cm^3); **D**, Resting bud scar number; **E**, Maximum number of branch orders.



plant after the haustorium had formed (cf. Dawson et al. 1990a). For *Nothofagus solandri* and *Podocarpus totara*, growth ring formation is known to be annual (Wells 1972; Norton 1984) and growth rings are distinct and easily counted. However, for *Chamaecytisus palmensis*, growth rings were at times indistinct and age estimates for this species may be less accurate than for the other two. Although New Zealand mistletoes show no winter growth (Powell & Norton 1994), growth rings are indistinct in the three mistletoe species studied here (Patel 1991) and could not be used to age the mistletoes.

For each of the three mistletoe species simple linear regression (using S-PLUS software; MathSoft 1995) was used to test for the correlation of anatomically estimated age with each of the five non-destructive measures. In addition, a multiple regression was calculated for age against all five measures (for each species). Before fitting the regressions, age and all five potential estimators of age were tested for the regression's assumption of normality. As a result of these tests, age, host branch diameter, mistletoe diameter, and mistletoe volume were log-transformed

before analysis. Thus, the regressions for any combination of age and its estimators are in fact curvilinear (logarithmic or power curves). The extent of non-linearity was tested by fitting a power curve to each data set (logarithmic or power) and testing the estimate of slope (which corresponds to the power exponent) against a linearly expected value of one using a simple t-test ($t = (\text{slope}-1)/\text{SE}_{\text{slope}}$). The fitted equations and their confidence intervals were back-transformed for the purposes of graphing and estimation.

RESULTS

Predictions of anatomical mistletoe age using all five non-destructive predictors of mistletoe age are significant for all three mistletoe species, although the variance explained in the regressions shows considerable variation (Table 1, Fig. 1–3). Of the three mistletoe species, *Alepis flavida* age is best modelled by the non-destructive predictors with R^2 values ranging from 0.686–0.849 (Table 1, Fig. 1).

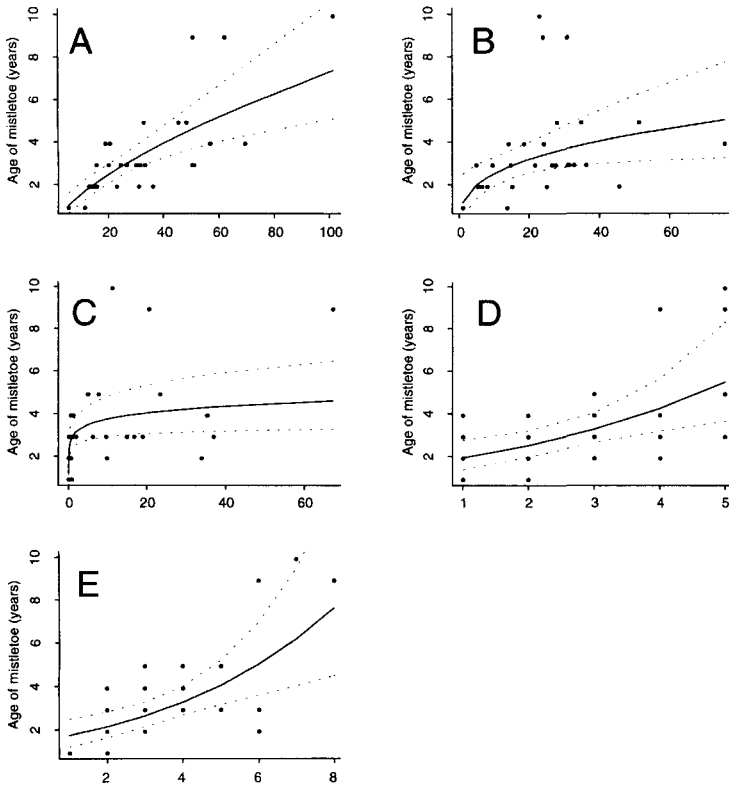


Fig. 3 The relationship between mistletoe age as determined by anatomical analysis and a number of non-destructive potential indices of age for *Tupeia antarctica*. The solid line is the fitted regression, with 95% confidence intervals indicated by the dotted lines ($n = 30$). **A**, Host branch diameter (mm); **B**, Mistletoe basal diameter (mm); **C**, Mistletoe volume (cm^3); **D**, Resting bud scar number; **E**, Maximum number of branch orders.

Table 1 Summary of the fit of the regressions (R^2 , $P(\text{regression})$) for predicting mistletoe age from non-destructive measurements for the three mistletoe species and results for test for non-linear regressions ($P(\text{non-linear})$).

Predictor	R^2	$P(\text{regression})$	$P(\text{non-linear})$
<i>Alepis flavida</i> (n = 26)			
Host branch diameter (log)	0.849	<0.0001	0.4506
Mistletoe basal diameter (log)	0.773	<0.0001	0.3083
Mistletoe volume (log)	0.796	<0.0001	<0.0001
Max. number of annual resting bud scars	0.719	<0.0001	0.0002
Max. number of branch orders	0.686	<0.0001	0.3452
Multiple regression (all predictors)	0.866	<0.0001	—
<i>Ileostylus micranthus</i> (n = 30)			
Host branch diameter (log)	0.790	<0.0001	0.1903
Mistletoe basal diameter (log)	0.602	<0.0001	0.1944
Mistletoe volume (log)	0.699	<0.0001	<0.0001
Max. number of annual resting bud scars	0.653	<0.0001	0.0470
Max. number of branch orders	0.545	<0.0001	<0.0001
Multiple regression (all predictors)	0.834	<0.0001	—
<i>Tupeia antarctica</i> (n = 30)			
Host branch diameter (log)	0.622	<0.0001	0.0013
Mistletoe basal diameter (log)	0.295	0.0019	<0.0001
Mistletoe volume (log)	0.352	0.0006	<0.0001
Max. number of annual resting bud scars	0.445	<0.0001	0.0885
Max. number of branch orders	0.384	0.0003	0.0061
Multiple regression (all predictors)	0.688	<0.0001	—

Ileostylus micranthus age is next best modelled with R^2 values ranging from 0.545–0.790. *Tupeia antarctica* age is the least well modelled of the three, with R^2 values ranging from 0.384–0.622.

For *Alepis flavida* and *Ileostylus micranthus*, only mistletoe volume and resting bud scar number (*Ileostylus* only) regressions were significantly non-linear (Table 1); the other regressions were all linear. However, for *Tupeia antarctica*, all regressions except for the number of resting bud scars were significantly non-linear (Table 1).

Diameter of the host branch is the best predictor of mistletoe age in all three species, with R^2 values ranging from 0.622–0.849 (Table 1). For *Alepis flavida* and *Ileostylus micranthus*, volume is the next best predictor ($R^2 = 0.796$ and 0.699). However, for *Tupeia antarctica*, the maximum number of nodes is the second best predictor ($R^2 = 0.445$). Maximum number of branch orders is the poorest predictor of age in *Alepis flavida* and *Ileostylus micranthus* ($R^2 = 0.686$ and 0.545), while mistletoe basal diameter is the poorest predictor for *Tupeia antarctica* ($R^2 = 0.295$). Although multiple regressions did provide significantly better predictions of mistletoe age than the simple regressions using the diameter of the host branch for each of the species, the increase in explained variance was relatively small ($R^2 = 0.866$ cf. 0.849 ; $R^2 = 0.834$ cf. 0.790 ; $R^2 = 0.688$ cf. 0.622).

DISCUSSION

Our results show that it is possible to predict mistletoe age using a range of non-destructive indices, of which host branch diameter immediately below the haustorium is the best (Table 1). Our results extend the earlier work of Dawson et al. (1990a) on the viscaceous mistletoe *Phoradendron juniperinum* to loranthaceous mistletoes and is, as far as we are aware, the first such application to loranthaceous mistletoes. These results are important as they provide a method that allows the study of population dynamics and other age-dependent aspects of mistletoe ecology without destructively sampling the mistletoe population. The only previous work with aging loranthaceous mistletoes (Reid & Lange 1988) was constrained by an inability to age the mistletoes anatomically.

Despite the good regression statistics (Table 1), it is important to recognise that the use of the regressions as predictive tools has a number of limitations. The 95% confidence limits (Fig. 1–3) show that all estimates of age do have an error associated with

them which varies between predictors and mistletoe species. This error increases with increasing age because of the small sample size and the effect of log-transformation used to deal with the small sample size. Furthermore, the non-linear nature of many of the regressions (Table 1) suggests further caution in extrapolating directly from the predictor to age outside of the sampled range. The best predictor of mistletoe age here (host branch diameter) is linear for *Alepis flavida* and *Ileostylus micranthus*, but is non-linear for *Tupeia antarctica*. However, several of the other predictors have strong non-linear relationships with mistletoe age reflecting the non-linear way in which mistletoes grow (see below) and the calculation of the variables themselves (e.g., mistletoe volume). The regression equations are also based on mistletoe-host pairs at one particular site and may not be applicable at other sites or on other host species for these same mistletoes. Differences in mistletoe and host growth rates between sites and host species are likely to be particularly important. This may be particularly significant for *Ileostylus micranthus* and *Tupeia antarctica* which parasitise a large number of host species (de Lange et al. 1997).

Of the three mistletoe species studied, the regression statistics are weakest for *Tupeia antarctica* (Table 1, Fig. 3). While it may be that age is more difficult to estimate for *Tupeia antarctica*, we suspect that our estimates of host branch age may not have been as good for this species as for the other host species because of occasional indistinct growth rings. As a result, our estimates of anatomical mistletoe age may have been in error which would have contributed to these poorer results.

We were unable to develop predictive models of mistletoe age for *Peraxilla colensoi* and *Peraxilla tetrapetala*, the other extant New Zealand loranth. This was because of the growth form of these species (Powell & Norton 1994; Norton et al. 1997). Typically in *Peraxilla tetrapetala*, plants establish on young host shoots and then produce runners which extend to reach the host trunk where they proliferate. Haustoria form at intervals along these runners, eventually resulting in very large and complex mistletoe clumps. In most cases it appears that the original mistletoe ramet dies when the branch to which it is attached dies. *Peraxilla colensoi* appears to have a similar growth architecture (Powell & Norton 1994). Because of this growth architecture, ramet age is not correlated with genet age, and hence it is not possible to estimate mistletoe age using the approach taken here with the other species.

Host branch diameter immediately below the

haustorium was constantly the best predictor of mistletoe age in our study. Reid et al. (1995) have suggested that this is because mistletoes almost always establish on young host branches (typically less than 20 mm in diameter; Reid 1991; Yan & Reid 1995) and because host branch diameter increases monotonically through time, unlike other variables such as mistletoe volume, branch number, and number of resting bud scars. A preference for establishment on small host branches also occurs in the mistletoe species studied here (e.g., *Alepis flavida*; D. A. Norton & J. J. Ladley unpubl. data). As noted by Reid et al. (1995), the number of branch orders and related measures were in general poorer predictors of mistletoe age, despite the highly significant results of Dawson et al. (1990a) using the number of branch bifurcation events as their predictor of *Phorodendron* age. The poorer performance of these predictors here is likely to be due to differences in growth architecture. In the three mistletoe species we studied, branch order and the number of annual resting bud scars are not monotonically related to age, with only small increases in these predictors with age in older mistletoes (e.g., Fig 1D, E). Powell & Norton (1994) noted that branching in *Alepis flavida* is slow and can vary with plant age. Furthermore, these variables together with mistletoe volume (see below) are also affected by factors unrelated to mistletoe age (e.g., browse and breakage), whose effects should cumulate with plant age leading to the non-linear responses observed here.

In previous studies we have used mistletoe volume as a proxy for age (Norton et al. 1997). While volume provides significant predictions of age for *Alepis flavida* and *Ileostylus micranthus*, the regressions are strongly non-linear, especially in larger mistletoes (Fig. 1C, 2C, 3C), suggesting caution in using volume. However, in many situations it is not possible to measure host branch diameter (e.g., when the mistletoe occurs high in the host tree) and in these situations mistletoe volume may be the only variable that can be readily assessed.

Although the relationships we have presented are based on single mistletoe-host pairs from single sites, our results do suggest that in future studies of the ecology of the three mistletoe species examined here, the diameter of the host branch immediately below the haustorial attachment can be used as a reliable indicator of mistletoe age and should be used where at all possible. However, information on host growth rates will be required to calibrate the relationship when applied at other sites. We see little need or justification for extensive destructive sampling of mistletoes in order to age individual plants.

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