

Flower predation by *Zelleria maculata* (Lepidoptera) on *Peraxilla* mistletoes: effects of latitude and fragmentation, and impact on fruit set

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Published on-line: 8 October 2008

Abstract: Flower predators (florivores) may affect plant reproduction directly through loss of pollen and ovules, or indirectly by deterring pollinators which avoid damaged flowers. Caterpillars of the widespread endemic moth *Zelleria maculata* feed inside flower buds of the endemic mistletoes *Peraxilla tetrapetala* and *P. colensoi* in New Zealand. We measured flower predation rates between 1995 and 2007 at 24 sites throughout New Zealand and assessed *Zelleria* feeding impact on fruit set. *Zelleria* predation showed a strong latitudinal gradient, being rare in the North Island but affecting 81% of flowers in 1996 at Waipori (Otago) and averaging > 38% at Waipori and Eglinton (Fiordland). The reasons for this pattern are unknown, but a wasp parasitoid of *Zelleria*, *Campoplex* sp. (Ichneumonidae), may be less common further south. Rates of *Zelleria* attack were higher in mistletoes growing several metres above ground and in less fragmented habitats (i.e. in shaded positions, and away from edges), and higher in *P. tetrapetala* than *P. colensoi*. An experiment following 1005 tagged *P. tetrapetala* flowers at two sites showed that *Zelleria* attack significantly reduces both pollinator-flower-opening rates (required for effective pollination) and fruit set rates. *Zelleria* attack increased the chance of a flower not being opened by pollinators from 11 to 37% at Ohau in 1996 (reducing fruit set from 28% to 7.9%) and flower non-opening from 6 to 30% at Craigieburn in 1995 (fruit set: 44.4% to 16.6%), representing reductions in fruit set of 72% and 63% at the two sites. Therefore, *Zelleria* reduces reproduction in *Peraxilla* spp. through both pollinator deterrence and direct loss of flowers at many sites. This could affect conservation of these mistletoes, which are seed-limited and in decline. However, *Zelleria* impact is reduced by its avoidance of edge habitat, as *P. tetrapetala* is more abundant and flowers more heavily on edges.

Keywords: edge effects; florivory; herbivory; Loranthaceae; parasitoid wasp; *Peraxilla colensoi*; *P. tetrapetala*; plant reproduction; pollination

Introduction

Florivory (herbivory on flower parts) is a key ecological interaction that can have a major impact on plant reproduction (Crawley 1989), yet in comparison with folivory or seed predation, florivory is relatively understudied and its impact not well understood or appreciated (McCall & Irwin 2006). Florivory appears to be common and affects a significant number of plant species both in New Zealand and globally (Dugdale 1975; Crawley 1989; McCall & Irwin 2006). Florivores can affect plant fitness in two ways – directly through damaging ovules and anthers and consuming plant resources, and indirectly by changing the attractiveness of plants to pollinators, thereby reducing pollen export and receipt. In some cases a large fraction of the flower crop can be damaged and the reproductive potential of the pollen and ovules

thereby is lost; for example, Weiss (1996) reported losses of >71% of flowers in *Centropogon solanifolius* in Costa Rica, and Breedlove and Ehrlich (1968) losses of 50–79% in *Lupinus amplus*. In addition to these direct losses of gametes, pollinator avoidance compounds the effects of florivory. For example, the pollen beetle *Meligethes rufimanus* halved the number of inflorescences in attacked *Isomeris arborea* plants, and in the surviving flowers reduced nectar production fourfold, pollen counts at least threefold, and pollinator visitation per flower threefold (Krupnick & Weis 1998; Krupnick et al. 1999). As a result, pollen export per undamaged flower was halved, and pollen deposition reduced (Krupnick & Weis 1999). These losses clearly reduce male and female fitness substantially and must create strong selective pressure on plants to avoid the damage. Despite the apparent ubiquity and importance of these kinds of interactions, McCall

and Irwin's (2006) review found few studies on floral predation, or on the multiple factors affecting it and its effects on plant fitness.

In New Zealand, one widespread florivore is the native moth *Zelleria maculata* (Yponomeutidae; hereafter *Zelleria*), whose caterpillars feed inside flower buds of the mistletoes *Peraxilla tetrapetala* and *P. colensoi* (Loranthaceae) in New Zealand (Patrick & Dugdale 1997). Outside the flowering season, the caterpillars feed on the leaves of both *Peraxilla* species, in early instars by leaf mining and in later instars by feeding externally. Both mistletoe species are endemic to New Zealand, growing primarily upon *Nothofagus* spp., have declined dramatically in density and range since 1840 (de Lange & Norton 1997), and are currently listed as 'gradually declining' (Hitchmough et al. 2007). Although the flower predator is native, plant seed production has decreased because of reduced bird densities from human impacts (Robertson et al. 1999), so additional factors reducing seed production assume greater importance. Hence, determining the effects of florivory on reproduction in *Peraxilla* spp. is of both theoretical and practical significance. As the moth is widespread and abundant, there are no corresponding concerns for its persistence.

Previous work has shown that *Zelleria* can reach high densities at some sites – at four South Island sites, Kelly et al. (2000) reported that 8–48% of *P. tetrapetala* flower buds were attacked by *Zelleria*. It also has a high impact, with Robertson et al. (2005) saying that *Zelleria*-attacked flowers rarely set fruit; as a result, a series of papers on pollination treatments and fruit set in *Peraxilla* spp. have presented data based only on flower buds not containing *Zelleria* (Robertson et al. 1999, 2008; Montgomery et al. 2003; Kelly et al. 2004, 2007). This is appropriate if the aim is to measure the interaction between bird pollinators and viable flowers, but obviously gives an incomplete picture of factors affecting overall reproductive success of the plants.

Floral herbivory by *Zelleria* also apparently interacts with other factors of importance to mistletoe reproduction. In New Zealand there has been extensive clearance of once-continuous native forest, and the ensuing fragmentation of habitat has been shown in *Peraxilla* and the closely related *Alepis flavida* to variously affect mistletoe adult density (Kelly et al. 2000), adult growth and survival (Bach & Kelly 2007), leaf herbivory (Bach & Kelly 2004a), pollination by birds and bees (Kelly et al. 2000; Montgomery et al. 2003; Burgess et al. 2006), fruit dispersal (Kelly et al. 2000; Bach & Kelly 2004b), and seedling establishment (Bach et al. 2005). Moreover, a study in the Lake Ohau area (central South Island) showed that *Zelleria* predation rates were much higher in a stand of continuous forest (48%) than in three nearby fragments of varying size (predation rates 8–15%; Kelly et al. 2000).

Globally, fragmentation usually has negative effects

on native plant reproduction (Burgess et al. 2006), but reproduction in *P. tetrapetala* benefits from fragmentation for three reasons. Pollination of *P. tetrapetala* by both birds and bees is more effective on edges (Montgomery et al. 2003; Burgess et al. 2006), mistletoe density is higher there (Kelly et al. 2000), and *Zelleria* apparently avoids edges as noted above for Lake Ohau. However, the Lake Ohau study was restricted to a limited area in a single season. Many questions remain about the range, density, and impact of *Zelleria* on *Peraxilla*.

In this study, to answer those questions, our aims were to (1) measure the levels and temporal variability of flower predation by *Zelleria* on *Peraxilla* mistletoes; (2) determine the spatial distribution of *Zelleria* flower predation, and the factors affecting it, at national, within-site, and within-plant levels; and (3) determine the impact of *Zelleria* flower predation on fruit set.

Materials and methods

Biology of *Peraxilla* spp., *Zelleria maculata*, and its parasitoid, *Camptoplex* sp.

Peraxilla spp. have explosive buds that require force on the tip of the bud to open normally (top-opened; Fig. 1), a process done easily for both species by bellbirds (*Anthornis melanura*) and tūī (*Prothemadera novaeseelandiae*) (Meliphagidae), and only on *P. tetrapetala* with difficulty by native bees (Ladley & Kelly 1995; Kelly et al. 1996). When flowers are not opened by a bird or bee within 5–7 days, the petals abscise basally while still joined together at their tips, and eventually pull off over the style as a unit complete with the anthers (bottom-opened; Fig. 1). Both birds and bees can be effective pollinators when they open flowers (Robertson et al. 2005), but where pollinators are excluded or at low densities, most flowers bottom-open, and such flowers have very low fruit set because the stigma is hidden inside the petal tips until late in the flower's life (Ladley & Kelly 1995; Kelly et al. 2004). Although these species are fully self-compatible, flowers that bottom-open inside pollinator exclusion bags set very little fruit, so delayed selfing seldom occurs when flowers open this way (Robertson et al. 1999; Kelly et al. 2007).

There are two species of *Zelleria* and both feed on mistletoe leaves, but apparently only *Z. maculata* also feeds on *Peraxilla* flowers (Patrick & Dugdale 1997). We reared adult moths from Ohau, Waipori and Eglinton (spanning both *P. tetrapetala* and *P. colensoi*) and these were identified as *Zelleria maculata* (J. Dugdale, pers. comm.), so we assume that *Z. maculata* was the flower predator at all sites.

Caterpillars of *Zelleria* feed inside *Peraxilla* flower buds, variously eating filaments, anthers, the style and/or the inside of the petals. Fully grown caterpillars leave the flowers to pupate on the mistletoe stems (Patrick & Dugdale 1997). Unopened flower buds damaged by

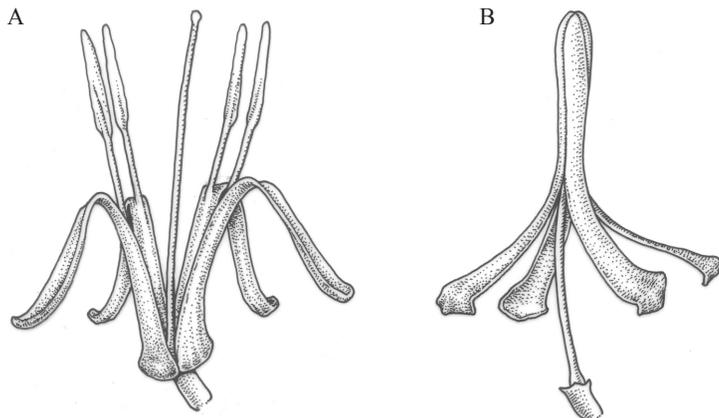


Figure 1. Opening modes of *Peraxilla* flowers (*P. colensoi*) is illustrated, mean bud length 47 mm). (a) Top-opened flowers, which have been opened from the petal tips by a pollinator. (b) Bottom-opened flowers which have not been opened by a pollinator, with petals abscising from the base late in the flower's life and pulling off as one unit over the style. The single-seeded inferior ovary is at the bottom of each drawing. Drawings by Tim Galloway.

Zelleria can be recognised by a small silked-up entrance hole and by dark frass inside the flower, which changes its colour externally. Top-opened flowers on the plant and fallen flower petals beneath it that have contained *Zelleria* show visible frass and chewing damage.

At Craigieburn and Ohau, we frequently saw small parasitoid wasps ovipositing into *P. tetrapetala* flowers presumed to contain *Zelleria* caterpillars. We reared adult wasps from pupae of *Zelleria*. The wasps (Fig. 2) have been deposited in the New Zealand Arthropod Collection and were identified as *Campoplex* sp. (Ichneumonidae; Jo Berry, pers. comm.). This cosmopolitan genus is in need of revision in New Zealand so our samples could not be identified to species level.

Measuring *Zelleria* predation rates

To estimate the overall percent of flowers damaged by *Zelleria* on a plant we first scored flowers remaining on the plant. These measurements were only made when flowers were ripe, as *Zelleria* predation is hard to recognise in unripe flower buds. On larger plants several branches were selected holding in total about 100 flowers, and these were visually scored as *Zelleria*-attacked or unattacked. On plants with fewer flowers we scored all flowers on the plant. We also recorded the percentage of flowers for which the petals or entire flower had already abscised (determined by the remaining ovary or pedicel scar). However, casual observation confirmed by subsequent analysis showed that *Zelleria*-attacked flowers are more

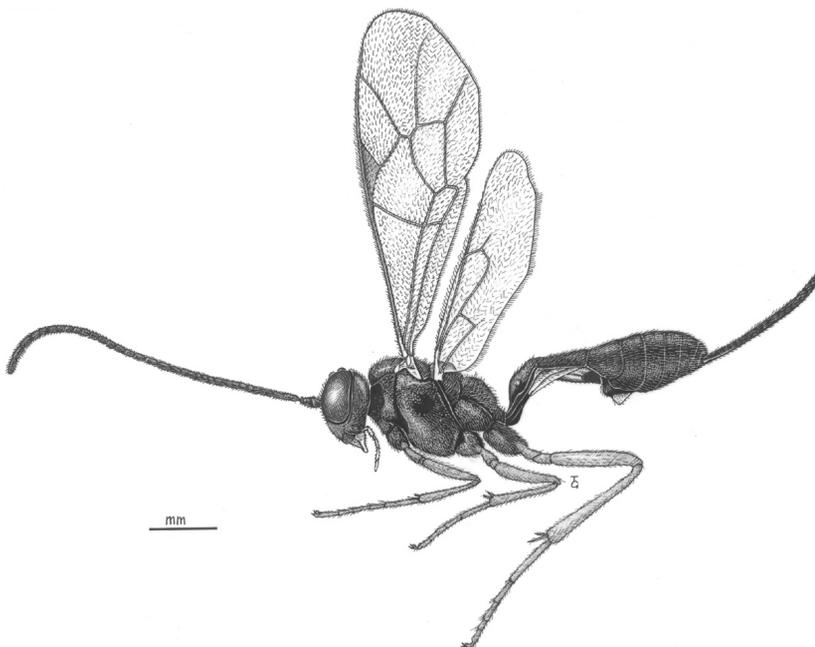


Figure 2. Parasitoid wasp (*Campoplex* sp.) that parasitises *Zelleria maculata*. Scale bar is 1 mm. Drawing by Tim Galloway.

likely to abscise entirely, so flowers remaining on the plant are disproportionately unattacked. We therefore also systematically collected 100–200 fallen flower petals from the ground directly underneath the mistletoe. These petals were also classified as coming from *Zelleria*-attacked or unattacked flowers. Then a weighted-average *Zelleria* predation rate for the plant was calculated as:

$$Z(A) = Z(P) \times (1-f) + Z(G) \times f$$

where $Z(A)$ = overall percent *Zelleria* attacked, $Z(P)$ = percent *Zelleria*-attacked flowers on the plant, $Z(G)$ = percent *Zelleria*-attacked flowers off the ground, and f = proportion of flowers that have fallen. For about 50 plants where we had $Z(P)$ but no data for $Z(G)$ and $0 < f < 0.05$ (so nearly all flowers were still on the plant) we set $Z(A) = Z(P)$ since $Z(G)$ would have had very little impact on $Z(A)$.

Three measures of forest fragmentation were recorded for each mistletoe plant: light environment, canopy cover, and the percentage of the host tree's canopy circumference that formed part of a forest margin rather than adjoining another tree. Light reaching the mistletoe was visually assessed from 10 for a mistletoe in direct sun all day to 1 for a plant in deep shade all day. Canopy cover was estimated visually as percentage ground cover within a 10-m radius of the mistletoe. We used these fragmentation scores to see if *Zelleria* predation rates were related to plant edge exposure. We also measured the height of the base of the mistletoe from the ground.

Locations used

We present information from 24 sites (Appendix 1) at 13 locations throughout New Zealand (Fig. 3). Sites within a location differed in degree of forest fragmentation. For example, in the Temple Valley near Lake Ohau we had three sites: Temple Gorge with mistletoes in a remnant forest patch with many canopy gaps, Temple Carpark 1.5 km to the northwest using mistletoes on tall trees on an exposed forest edge, and Temple North Branch 500 m further northwest inside continuous beech forest. In the Eglinton Valley, we did not analyse separate sites due to low numbers of available plants; *P. tetrapetala* was mainly sampled at Knobs Flat and *P. colensoi* mainly at Deer Flat (2 km to the south), but we also included in the Eglinton means some *P. tetrapetala* from Dore Track (3 km north) and Deer Flat, and some *P. colensoi* from Dore Track.

Our study includes data from the 1994/95 season (hereafter 1995) to the 2007 season (Appendix 1), encompassing 82 site-years and 1050 plant-years. The number of plants scored per site-year averaged 12.8 (range 1–54). At the Belgrove site there was only a single isolated *P. tetrapetala* plant in farmland, and at Temple Carpark we had complete *Zelleria* counts for only one plant in 1996 but 10 plants in 1998. We scored ≥ 4 plants in 67 of the 82 site-years. In total we examined 139 785 flowers on

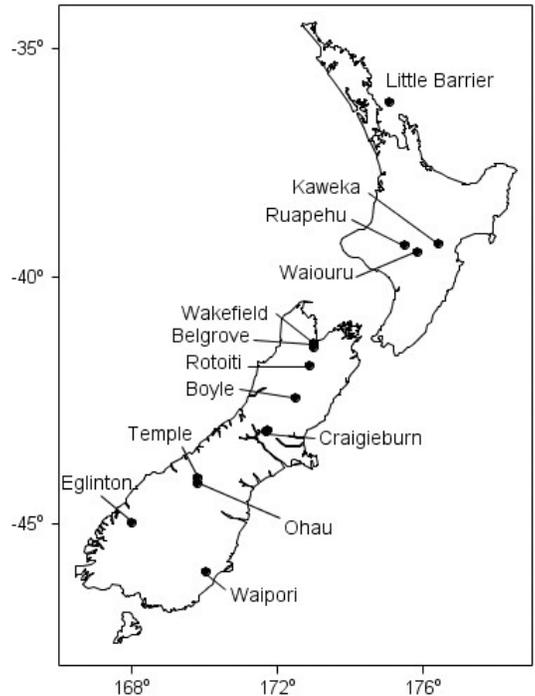


Figure 3. Locations used in the study. Some locations contained several sites varying in degree of forest fragmentation. See Appendix 1 for a full listing of sites.

plants for *Zelleria* predation, plus 135 956 flower petals collected off the ground.

Factors affecting *Zelleria* predation rates

We analysed *Zelleria* predation rates (percent of flowers containing *Zelleria*) on three levels. Across the whole country, site-mean *Zelleria* predation (mean of plant means for all available plants at the site) was predicted from latitude, and year (as a factor). The analysis used a Gaussian GLM and proportion *Zelleria* was arcsin-square-root transformed. A binomial GLM could not be used because the proportion *Zelleria* is a weighted mean of estimates off the plant and off the ground, as explained above.

At the plant level, we used a GLM for plants across all sites where the response variable was percent of flowers per plant attacked by *Zelleria* (again arcsin-square-root transformed), and predictors were plant height above the ground, plant fragmentation scores, and *Peraxilla* species (*colensoi* or *tetrapetala*). In this analysis latitude and year were fitted first as covariates.

Because of limitations of access using ladders, most of our mistletoe plants at all sites were near the ground. Only one of 1050 was above 4.3 m height, whereas the

canopy is typically at 10–20 m. To more fully explore any height effects, we used information on 35 plants higher in the canopy (mean 6.7 m height, maximum 13.4 m) on 11 host trees at Broken River in 1998 accessed using climbing ropes (Robertson et al. 2008). For analysis these ‘vertical transect’ plants were combined with the 61 plants from nearer the ground at this site in 1998 used in the all-sites analysis. Because there were several vertical-transect plants per host tree we could not estimate each plant’s *Zelleria* predation rates from flowers off the ground, preventing us from calculating an overall *Zelleria* predation rate for these plants. Therefore this analysis used only the percent of flowers still on the plants that contained *Zelleria*, which is usually slightly lower than the overall rate, and analysed the relationship with height using CoStat version 3.1 (Cohort Software, Monterey, California).

To test for factors operating at the within-plant level, we measured 99 branches on 66 plants at Ohau (Round Bush and Parsons Creek) in the 1998 season. Branches were selected to be either on the lower part of the mistletoe proximal to the ground (basal), or on the upper part with other foliage between the branch and the ground (not basal). Some branches were classified as partly basal, and due to restricted access only single branches were able to be used on some trees. The height of the mistletoe’s base above the ground, the height of the branch above the base of the mistletoe, and the type of ground cover (leaf litter, gravel, or a mixture of the two) was also recorded. On each branch about 100 flowers were examined and scored for the presence of *Zelleria*. Analysis used a binomial GLM with branch position, mistletoe height, branch height and ground cover as predictors.

Effects of *Zelleria* on fruit set and flower opening rates

To determine the effect of *Zelleria* on fruit set, we tagged and followed individual flowers of *P. tetrapetala* to fruit set at two locations. At Ohau (Round Bush) in the 1997 season we tagged 44 branches on 10 flowering plants. Each branch was randomly allocated to one of two treatments: open (unmanipulated) or caged (enclosed in 11-mm-mesh wire to exclude bird pollinators, to test for interactions between pollinator abundance and *Zelleria* predation). Branches typically carried 20–50 flower buds and we tagged enough branches to have about 100 buds of each treatment on each plant. Each flower on a branch was tagged with coloured wire and observed daily from 12 to 22 December 1996 and again on 28 December to record whether flowers were *Zelleria*-attacked or not, and whether they were top-opened or bottom-opened (see Fig. 1 and Methods section). Fruit set was recorded on 13 March 1997. In total there were 1288 flowers on the tagged branches, but we excluded any flower that was already open on 12 December or had not yet opened by 28 December, and also a few flowers that were damaged by chaffinches (*Fringilla coelebs*) or wind-opened by rubbing against the sides of the cages. This left 633 flowers whose

fates were known. We used a binomial GLM to estimate fruit set from the predictors *Zelleria* attack, caged/not, and top/bottom opened, in a split-plot design. The unit of replication in the GLM was the branch \times top/bottom-opened combination (e.g. all top-opened flowers on a single branch were one replicate). We also tested whether *Zelleria* attack and caged/not predicted the chance of a flower being top-opened versus bottom-opened.

The same experiment was set up at Craigieburn (Broken River) in the 1996 season, although with fewer flowers. We mapped flowers on 58 branches on eight plants and applied cages to half the branches. Flowers were mapped between 5 and 9 January 1996 and followed every day for 10 days. Fruit set was scored in March 1996. The tagged branches carried 1159 flowers. After excluding those that opened before the start or after the finish, and also excluding some harvested for pollen tube analysis, we were left with 372 flowers for analysis, using the same methods as for Ohau.

Results

Factors affecting abundance of *Zelleria*

The analysis of mean *Zelleria* predation at the site level showed a strong effect of latitude ($F_{1,69} = 42.86, P < 0.001$) but no significant effect of year ($F_{11,69} = 1.40, P = 0.19$). Flower predation increased markedly further south (Fig. 4), with *Zelleria* always <3% in our North Island samples but peaking at 81.05% of flowers on *P. colensoi* at Waipori in 1996. There was wide variation among years (Appendix 1), but no significant year effect because changes from year to year were inconsistent across locations. This is illustrated by the fact that none of the three sites with the longest runs of data had significantly correlated levels of *Zelleria* predation across years (Broken River versus Gullerys, $n = 10, r = 0.46, P = 0.18$; Gullerys versus Round Bush, $n = 8, r = -0.21, P = 0.61$; Broken River versus Round Bush, $n = 8, r = -0.34, P = 0.41$).

At the plant level, after allowing for latitude and year there were significant effects of plant height, all three measures of fragmentation, and mistletoe species (Table 1). The coefficients show that *Zelleria* predation was higher for mistletoes that were higher off the ground, were in more shady places, had less of their host tree’s canopy abutting edge rather than forest, and were in areas with less canopy cover within 10 m. After allowing for all the above factors, *P. tetrapetala* had significantly higher *Zelleria* predation than *P. colensoi*.

The height effect appears contradictory to the shade effect, as increased height means plants will eventually reach the canopy and be exposed to high light. However, all but one of the plants analysed in Table 1 were within 4.3 m of the ground. The height effect over a wider range of mistletoe heights was checked at Broken River in 1998, although restricted to on-plant *Zelleria* rates rather

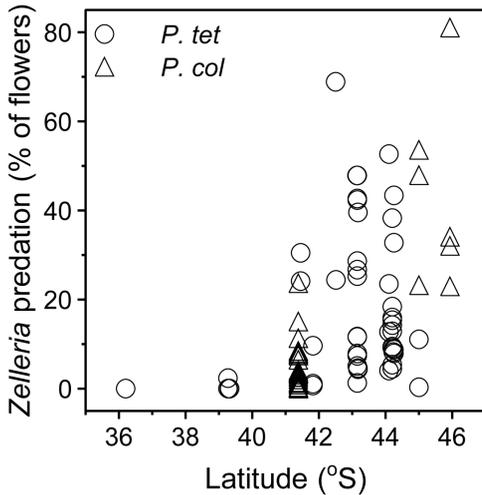


Figure 4. Percent of *Peraxilla* flowers predated by *Zelleria* versus latitude for *P. colensoi* (triangles) and *P. tetrapetala* (circles). Each symbol represents the mean for one site in one year, with multiple years at some sites (see Appendix 1). The effect of latitude was highly significant (see text).

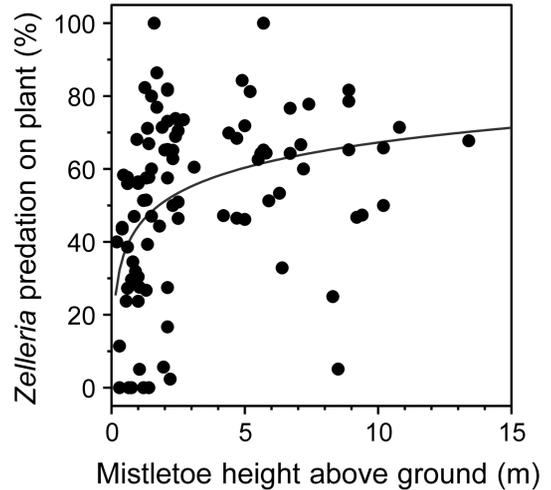


Figure 5. Effect of height on *Zelleria* predation of *Peraxilla tetrapetala* flowers (percent of flowers still on the plant that were attacked) at Broken River, 1998. The regression was highly significant ($n = 96$, $F_{1,94} = 18.28$, $P < 0.001$, $y = 44.27 + 9.987\ln(x)$).

than overall rates. There was a significant, but complex, relationship (Fig. 5): *Zelleria* rates increased initially as in the previous analysis, but above about 5 m they flattened off. When analysing only the 35 plants on the vertical-transect trees (nearly all > 4 m height), *Zelleria* rates **decreased** significantly with height (binomial GLM with host tree as a block effect, height effect $F_{1,23} = 16.77$, $P < 0.001$) but with a very shallow slope. Hence we conclude that *Zelleria* predation is lower within a few metres of the ground, but reaches a plateau in the middle and upper layer of the forest. Note that because of restrictions of access by climbing ropes, all the mistletoes

on the vertical transects were at least 2 m below the top of their respective hosts.

At the branch level within Ohau (Round Bush and Parsons Creek sites) in 1998, there were significant effects of branch position ($F_{2,92} = 4.38$, $P = 0.015$) and height of mistletoe above the ground ($F_{1,92} = 7.93$, $P = 0.006$). Higher mistletoes again had more *Zelleria*, while branches not basal (i.e. with foliage between the branch and the ground) had less *Zelleria* than those half or fully basal. Height of the branch within the mistletoe, and nature of the ground cover, were both non-significant ($F_{1,92} = 0.05$, $P = 0.80$, and $F_{2,92} = 0.25$, $P = 0.78$, respectively).

Table 1. Analysis of plant-level *Zelleria* flower predation rates across all sites. Latitude and year are included as block effects but are not tested for significance at the plant level (see text for analysis at site level). Predation rates were arcsin-square-root transformed before analysis in a Gaussian GLM. For significant effects (in bold type), the fitted coefficient is given (units are arcsin-square-root transformed) with the mean and range of the predictor. For species, the coefficient is the increase in predation in *Peraxilla tetrapetala* over *P. colensoi*.

Factor	d.f.	Deviance	<i>F</i>	<i>P</i>	Coefficient	Mean (range)
Latitude	1	9.852	n/a			
Year	11	15.434	n/a			
Height	1	0.817	11.84	<0.001	0.0446	1.625 (0–6)
Sun	1	0.568	8.23	0.004	–0.0066	5.08 (2–10)
Tree edge	1	0.378	5.47	0.019	–0.0015	36.3 (0–100)
Canopy	1	0.345	4.99	0.026	–0.0017	50.6 (5–90)
Species	1	0.411	5.95	0.015	0.0570	
Residual	985	68.035				

Table 2. Effect on fruit set in *Peraxilla tetrapetala* of *Zelleria* attack, from split-plot binomial GLM analyses. Significant effects are in bold type.

(a) Ohau, 1997 season (i.e. summer 1996/97)

Predictor	d.f.	Deviance	<i>F</i>	<i>P</i>	Error MS
Plant	9	40.925	2.85	0.013	Branch
Cage	1	2.019	1.27	0.269	Branch
Branch	33	52.655			
<i>Zelleria</i>	1	25.268	31.86	<0.001	Residual
Top/bottom	1	7.157	9.02	0.003	Residual
Top/bottom × cage	1	5.924	7.47	0.007	Residual
<i>Zell</i> × cage	1	0.243	0.30	0.581	Residual
<i>Zell</i> × top/bottom	1	2.309	2.91	0.091	Residual
<i>Zell</i> × cage × top/bottom	1	0.299	0.37	0.540	Residual
Residual	88	67.937			

(b) Craigieburn, 1996 season

Predictor	d.f.	Deviance	<i>F</i>	<i>P</i>	Error MS
Plant	7	31.600	4.05	0.001	Branch
Cage	1	2.625	2.36	0.131	Branch
Branch	49	54.563			
<i>Zelleria</i>	1	17.513	41.90	0.0000	Residual
Top/bottom	1	2.708	6.48	0.0136	Residual
Top/bottom × cage	1	0.151	0.36	0.5500	Residual
<i>Zell</i> × top/bottom	1	0.022	0.05	0.8213	Residual
<i>Zell</i> × cage	1	2.939	7.04	0.0103	Residual
<i>Zell</i> × cage × top/bottom	1	0.000	0.001	0.9981	Residual
Residual	58	27.398			

Table 3. Fruit set (%) in *Peraxilla tetrapetala* by treatment. Cells give mean fruit set (with number of flowers), and the ratio of *Zelleria* fruit set over unattacked fruit set, for two locations. Dashes indicate that there are too few data to present a mean.

Treatment	Ohau			Craigieburn		
	Unattacked	<i>Zelleria</i>	Ratio	Unattacked	<i>Zelleria</i>	Ratio
Uncaged, top-open	29.7 (165)	7.21 (97)	0.24	46.4 (97)	19.2 (47)	0.41
Uncaged, bottom-open	14.3 (28)	8.9 (56)	0.62	– (1)	10.5 (19)	–
Caged, top-open	27.0 (111)	7.4 (27)	0.27	34.1 (85)	5.9 (34)	0.17
Caged, bottom-open	6.7 (75)	1.4 (74)	0.20	14.3 (49)	5.0 (40)	0.35

Effect of *Zelleria* on fruit set and flower opening rates

The GLMs gave very good fits to the fruit set data with 67% and 80% of the deviance explained at Ohau and Craigieburn respectively. As it was a split-plot design we tested Plant and Cage against the Branch error mean square, and other terms against the residual mean square (Table 2). At Ohau there were significant effects of plant, *Zelleria*, and top/bottom opening on fruit set, and one significant interaction (top/bottom × cage). The same effects were significant at Craigieburn except that a different interaction (*Zelleria* × cage) was significant. Therefore, *Zelleria* attack and the top- versus bottom-opened status of flowers, in interaction with the caged/uncaged treatment, affected

fruit set of flowers. Means of fruit set separated out by these factors (Table 3) showed that fruit set was always reduced in flowers that were bottom-opened rather than top-opened, and was reduced inside cages that excluded bird pollinators but allowed bees (although this last was not significant as a main effect). On top of these effects, *Zelleria* universally depressed fruit set, but not to zero. The relative fruit set in flowers with and without *Zelleria* varied with the other two factors but was usually around 0.2–0.35 (i.e. a reduction of 65–80% with *Zelleria*), although for uncaged bottom-opened flowers at Ohau the ratio was 0.62 (a 38% reduction).

The overall reduction in fruit set in the field caused by *Zelleria* is best estimated by the uncaged treatment, but depends on the fraction of flowers that are top-opened versus bottom-opened, which is itself affected by *Zelleria* attack. At both locations, the GLMs showed that top-opening rate was significantly affected by *Zelleria* (Table 4) as well as by the cage treatment. The percentage of flowers bottom-opening always increased for flowers containing *Zelleria*: at Ohau in cages from 41.4% to 72.3% and outside cages from 11.2% to 37.3%; at Craigieburn in cages from 35.6% to 57.8% and outside cages from 6.1% to 30.0%.

Combining the two *Zelleria* effects of increasing the fraction bottom-opening (which have lower fruit set) and lowering fruit set directly, the weighted mean fruit set for uncaged flowers decreased at Ohau from 28.0% of unattacked flowers making a fruit to 7.9% of attacked ones, a reduction of 72%. At Craigieburn there were insufficient data for uncaged bottom-open flowers without *Zelleria*, so we used an estimate of 14.3% fruit set (the figure from uncaged bottom-open unattacked flowers at Ohau and also for caged bottom-open unattacked flowers at Craigieburn). Including that estimate, the weighted mean fruit set for uncaged flowers at Craigieburn was 44.4% without *Zelleria* and 16.6% with it, a decrease of 63%.

Hence, *Zelleria* has two effects on flowers of *P. tetrapetala*: it reduces the likelihood of being top-opened (which is important for pollen to reach the stigma) by a factor of between 1.5 and 5 times, and decreases the chances of it setting fruit, usually by at least two-thirds. However, fruit set from flowers attacked by *Zelleria* is not zero.

Discussion

In the terminology of McCall and Irwin (2006) *Zelleria* is a generalist herbivore, in that the caterpillars can also feed on leaves. However, while flower predation was very evident at our sites in the flowering season, leaf herbivory was inconspicuous. A previous study over three sites of leaf herbivory in *Peraxilla* and *Alepis* found that leaf area loss to invertebrates generally was low and plants appeared resilient to it (Sessions & Kelly 2001).

When feeding in flowers, *Zelleria* can be classified as a pure florivore (McCall & Irwin 2006) because it only eats floral parts and does not directly damage the developing ovary or seed. This is probably partly because the ovary is inferior, well below the site of caterpillar feeding. This does lead to the question of which floral parts *Zelleria* feeds upon, given that up to 15% of *Zelleria*-attacked flowers do set fruit. In some flowers the caterpillars are observed to chew through the style, which seems likely to prevent fertilisation; however, in other flowers the style was undamaged. It may be that the developing anthers are a major target of the caterpillars; many species overseas specialise in feeding on anthers, which are relatively nutritious (McCall & Irwin 2006). An important point here is that our measure of *Zelleria* impact is solely based on maternal fitness (seed production). *Zelleria* almost certainly also reduces male fitness because it reduces flower-opening rates (thus reducing pollen export) and may also eat anthers (thus reducing pollen production). Such fitness costs would be additional to those we present.

Even so, the maternal fitness costs are substantial. Even allowing for the fact that *Zelleria* feeding in a flower

Table 4. Effect of *Zelleria* attack on *Peraxilla tetrapetala* flower top-opening rates (i.e. chance of a flower being opened normally before the petals abscise). Significant effects are in bold type.

(a) Ohau, 1997 season

Predictor	d.f.	Deviance	<i>F</i>	<i>P</i>	Error MS
Plant	9	78.846	5.03	<0.001	Branch
Cage	1	69.328	39.79	<0.001	Branch
Branch	33	57.503			
<i>Zelleria</i>	1	57.557	54.59	<0.001	Residual
Residual	647	630.077			

(b) Craigieburn, 1996 season

Predictor	d.f.	Deviance	<i>F</i>	<i>P</i>	Error MS
Plant	7	120.32	11.89	<0.001	Branch
Cage	1	12.11	8.37	0.006	Branch
Branch	48	69.42			
<i>Zelleria</i>	1	26.58	30.38	<0.001	Residual
Residual	350	282.24			

reduces fruit set by only two-thirds rather than totally, the high infestation rates at some sites mean that the overall reduction in fruit set (which equals seed production as there is a single seed per ovule) can be high. Assuming two-thirds of *Zelleria* flowers are destroyed, then *Peraxilla* seed crops nationally were reduced by about 10.5% (mean from all 82 site-years in our study). The mean estimated loss for the South Island south of Lewis Pass was 16%, and for south of Lindis Pass was 23%. The worst-affected site with multiple years of data was Waipori where over four years, seed lost because of *Zelleria* is estimated at 28% of the total. Clearly in southerly sites, the maternal fitness costs are very large.

An interesting question is to what extent the effects of *Zelleria* on fruit set are direct (from feeding damage) versus indirect (effects via pollinators; McCall & Irwin 2006). The decreased rates of top-opening in *Zelleria*-attacked flowers cause major reductions in fruit set rates (Table 3). Decreased top-opening may reflect both direct and indirect effects of *Zelleria*. Direct effects on top-opening are certain, because when trying to hand-open ripe flowers, those containing *Zelleria* are much harder to spring, presumably because of feeding causing changes in the turgor pressure that drives the opening mechanism. However, we hypothesise that *Zelleria*-attacked flowers are probably also less likely to have pollinating birds attempt to open them. The birds forage by visual colour cues to identify ripe buds. Videos of foraging birds show they move very quickly: tūi open ripe *P. colensoi* flowers in 0.23 ± 0.06 (SEM) seconds ($n = 23$; Ladley & Kelly 1995), the total flower handling time including inserting the beak and drinking the nectar is only 1.00 ± 0.13 seconds, and the time to select and reach another ripe flower on the branch averaged 0.59 ± 0.14 seconds. In our observations birds rarely tweak the end of a flower that does not readily open, which suggests not only that they can tell ripe from unripe buds, but also that they can probably distinguish between attacked and unattacked flowers. The visual cues are certainly evident to us. If so, *Zelleria* reduces fruit set both by making flowers harder to open, and by discouraging birds from opening them. An interesting corollary is that *Zelleria* also reduces the food availability for native birds, by reducing both mistletoe nectar and fruit. Both these food sources, when available, are important and highly preferred foods of bellbirds (Murphy & Kelly 2001).

Perhaps the strongest pattern we found in *Zelleria* predation is the latitude effect, with highest predation rates further south. The reasons for this are unknown, but our observations of the *Campoplex* parasitoid wasp are suggestive. The wasps were frequently seen at Ohau and Craigieburn (central South Island), but despite careful searching in suitable weather conditions we could not find any at Waipori and Eglinton (both in the far south). At the other sites we have not noted *Campoplex* but did not search for it. If the wasp is less common further south, this may

free *Zelleria* from regulation by its parasitoid, allowing caterpillar densities to increase and causing more flowers to be lost. Although this chain of events is speculative, other parasitoid wasps often appear to affect the density of their host insects (Kidd & Jervis 1997; Sadof & Snyder 2005; Evans et al. 2006), which is the basis of their use in biological control programmes. Clearly, more investigation of this parasitoid would be worthwhile.

Another topic worthy of closer investigation is the mechanisms for the smaller scale variation in *Zelleria* predation rates. *Zelleria* predation was higher on flowers away from edges, further above the ground, and basal in the mistletoe. These trends may be to do with flight and oviposition preferences of the moths, either because of direct habitat preference, or speculatively because of moths choosing sites with lower risks of parasitism. Detailed study of parasitism rates would be most enlightening. The analysis showed that *P. tetrapetala* had higher levels of predation than *P. colensoi* after allowing for latitude and other site factors, but the reasons are unknown. *P. colensoi* has larger flowers, which are stiffer and harder to open (Robertson et al. 2005), so it is possible that mechanical constraints make it harder for caterpillars to enter the buds.

Our final point is that forest fragmentation is confirmed to reduce flower predation in *Peraxilla*, as first suggested by Kelly et al. (2000). *Zelleria* reaches higher densities in the interior of forest, and on mistletoe plants in lower light and on host trees not on edges. Hence, fragmentation of native forest into a series of patches with much edge habitat has actually benefitted the mistletoes to some extent, with lower flower predation as well as higher visitation rates by both bird (Montgomery et al. 2003) and insect pollinators (Burgess et al. 2006) all leading to higher fruit set rates. This raises the interesting question of whether the native flower predator or the human-induced reductions in pollinator density (Robertson et al. 1999, Kelly et al. 2004) are having a greater effect on current levels of seed production in these declining endemic mistletoes. The answer will depend on the interactions among forest fragmentation, flower predation rates, and pollinator behaviour, which illustrates the complexity of factors affecting seed production in plants.

Acknowledgements

We thank Chantelle Waters for rearing *Zelleria maculata* and *Campoplex* adults, John Dugdale and Jo Berry for insect identifications, Tim Galloway for line drawings, and Ashley Sparrow for statistical advice. We also thank Phil Battley, Anne-Catherine Brunner, Andy Burgess, Vic Burgess, Deirdre Hart, Kirsten Kusak, Marina Manning, Shannon McDaniel, Krista McKinzey, Susannah Roddick, Laura Sessions, Stan van Uden, and Chantelle Waters for help with fieldwork. We thank the Department of

Conservation and other landowners for site access. Two anonymous referees gave helpful comments on the manuscript. The Public Good Science Fund provided funding under contract CO9X0004.

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