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Effects of mistletoe (*Phoradendron villosum*) on California oaks

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Mistletoes are a widespread group of plants often considered to be hemiparasitic, having detrimental effects on growth and survival of their hosts. We studied the effects of the Pacific mistletoe, *Phoradendron villosum*, a member of a largely autotrophic genus, on three species of deciduous California oaks. We found no effects of mistletoe presence on radial growth or survivorship and detected a significant positive relationship between mistletoe and acorn production. This latter result is potentially explained by the tendency of *P. villosum* to be present on larger trees growing in nitrogen-rich soils or, alternatively, by a preference for healthy, acorn-producing trees by birds that potentially disperse mistletoe. Our results indicate that the negative consequences of *Phoradendron* presence on their hosts are negligible—this species resembles an epiphyte more than a parasite—and outweighed by the important ecosystem services mistletoe provides.

1. Introduction

Mistletoes are a widespread group of plants whose negative effects on tree growth and mortality—often by increasing the sensitivity of their hosts to stress—are well established [1–3], historically prompting calls for their removal [4]. Such negative consequences are not universal, however. In particular, adverse effects have sometimes only been detected in severely infested trees [4,5], and although some mistletoes derive significant carbon from their hosts [6,7], this is not always the case [3,8]. These contradictory findings are in part due to differences among mistletoe taxa. Dwarf mistletoes, *Arceuthobium*, for example, derive much of their photosynthate from their conifer hosts, whereas *Viscum* and the American mistletoes, *Phoradendron*, are primarily water and mineral parasites, tapping into the xylem of their hosts and deriving the majority of their carbon from their own photosynthesis [1,9].

Despite the considerable research done on the physiological effects of mistletoe, we are aware of only two studies investigating their effects on host reproduction. Weir [10] reported significantly decreased germination in the seeds of trees infected by *Arceuthobium* in the northwestern USA, while Korstian & Long [4] reported a significant decline in both the quality and quantity of seeds produced by *Pinus ponderosa* that were parasitized by *Arceuthobium*.

Here, we report the effects of the Pacific mistletoe, *Phoradendron villosum*, on three species of California oaks (genus *Quercus*). We quantify growth, mortality, and seed production over a period of 10–38 years. Such long-term study is necessary because of the longevity of oaks, which can live hundreds of years, and their highly variable and synchronized seed production (masting behaviour) [11]; electronic supplementary material, figures S1 and S2).

2. Methods

We quantified *P. villosum* presence on three deciduous hosts [12], the valley oak *Quercus lobata*, blue oak *Quercus douglasii*, and California black oak *Quercus kelloggii*, at sites throughout California (electronic supplementary material, table S1). All trees were mature and initially selected for a study of mast seeding [13]. Trees were tagged and visited each autumn, at which time their acorn production was estimated by means of visual surveys during which two observers counted as many acorns as possible in 15 s [14]. Counts were summed and ln-transformed (ln(acorns counted in 30 s + 1)) to reduce non-normality. Surveys were conducted each year, starting when trees were selected and continuing until 2017, yielding 10–38 years of data.

Trees were measured by diameter at breast height (DBH) taken during the initial years of the survey at each site. For analysis of survivorship, we assessed the status of each tree (dead or alive) during acorn surveys based on the presence of green leaves. All trees considered to have died either fell or remained standing but exhibited no new growth; survivorship analyses excluded trees killed by fire or that had been cut.

Mistletoe abundance was assessed during three of the annual acorn surveys. The first, in autumn 1990, was prior to inclusion of most sites and performed only at Hastings Reservation and Jasper Ridge Biological Station. All sites were surveyed for mistletoe in 2001 and 2016. Abundance was quantified as the number of clumps of mistletoe present in the canopy of the tree. Because mistletoe presence was relatively stable through time (see Results), for analysis we either averaged the number of mistletoe clumps counted on trees across all surveys or divided trees into those with mistletoe during any survey and those without mistletoe. Although we did not estimate the volume of mistletoe, there is a strong correlation between number of mistletoe clumps and mistletoe volume (Pearson $r = 0.83$, $t = 79.0$, $N = 2878$, $p < 0.001$) based on our analysis of data on *Q. lobata* and *Q. douglasii* collected as part of a separate study at and near Hastings Reservation [15].

Data for additional analyses of factors correlating with mistletoe presence on hosts were gathered at Hastings Reservation. These included: (a) annual radial growth using dendrometers placed on each tree in 1994 and measured annually thereafter; (b) soil nitrogen (N) and soil phosphorus (P) availability estimated by means of four ion-exchange resin bags placed under each tree at a depth of 5–10 cm between October 1992 and April 1993 [16]; and (c) an estimate of water availability based on predawn xylem water potential (XWP) measured using a pressure bomb in September 1991 and 1994–1998 [17]. XWP varies depending on annual rainfall, but differences among trees are concordant among years [18], and thus we restricted our analyses to data collected in 1991, when all trees were measured.

Statistical analyses were conducted in R v. 3.3.1 [19]. For testing the effects of mistletoe on reproduction, we conducted a linear mixed-effects model ('lmer' in package 'lme4') with acorn production as the dependent variable, the mean number of mistletoe clumps present on each tree, DBH, 'species', and the interactions between 'species' and mistletoe abundance as explanatory fixed effects. 'Year' and 'tree ID within locality' were included as random effects. DBH was included as a fixed effect as the best available proxy for tree age; highly significant spatial synchrony [20] justified including 'year' as a crossed random effect. Analysis of the effects of mistletoe on radial growth used the same fixed and random effects except for locality, which was omitted as all trees with radial growth data were from Hastings Reservation.

For survivorship, we divided trees into those that survived from the initial year of sampling through to 2017 and those that did not. Differences in survivorship were tested by means of a generalized mixed model using a binomial error structure

and a 'logit' link function. Explanatory fixed effects included the mean number of mistletoe clumps, DBH, 'species', and the interactions between 'species' and mistletoe clumps; 'locality' was included as a random effect. The relationship between presence of mistletoe and water availability, soil nutrients, and tree size was assessed using general linear models with a binomial error structure and a 'logit' link function where presence/absence of mistletoe was the dependent variable and the independent variables included host species, the ecological variable of interest (the 'focal independent variable', or FIV), and the interactions between 'species' and the FIVs.

Overall species interaction effects were tested using log-likelihood tests ('lrtest' in package 'lmtest'). When significant, interactions were examined pairwise via *post hoc* tests with Tukey's correction.

3. Results

We surveyed 667 trees divided among 27 populations (electronic supplementary material, table S1); sites ranged from 42 to 950 km apart. Of the trees, 212 (32%) were surveyed for mistletoe during all three censuses and 331 (50%) were surveyed in 2001 and 2016. Of the 667 trees, 159 (24%) harboured mistletoe during at least one of the three surveys (electronic supplementary material, figure S3). Mistletoe abundance was generally moderate, with the mean number of clumps among trees with mistletoe ranging from 2.66 (maximum = 15) for *Q. lobata* to 7.03 (maximum = 27.5) in *Q. kelloggii* (electronic supplementary material, figure S4). The presence of mistletoe was stable across census years (Kendall's coefficient of concordance $W = 0.79$, $\chi_2 = 498$, d.f. = 2, $p < 0.001$).

Controlling for tree size, there was an overall significant, positive relationship between the mean number of mistletoe clumps and acorn production, whereas we found no relationship between mistletoe and either radial growth or survivorship (table 1). Overall, 93.6% of trees without mistletoe survived over the study period compared with 95.0% of trees that harboured mistletoe. None of the interactions was significant based on log-likelihood tests; however, there was a tendency for the relationship between acorn production and mistletoe to be lowest in *Q. douglasii*.

Of the ecological factors tested, larger trees with higher soil N were more likely to harbour mistletoe, although the latter effect was not quite significant (table 2). The effect of soil N was strongest in *Q. kelloggii* based on the negative interactions between 'species' and soil N for the other two species compared with *Q. kelloggii*, although the interaction effects were not significant in *post hoc* tests with Tukey's correction.

4. Discussion

Mistletoe is generally acknowledged to have both positive and negative ecosystem effects. On the positive side, mistletoe enhances biodiversity, generates nutrient-enriched litter, provides an important structural element in forest canopies, and its seed, flowers and leaves provide food for a variety of animals [21]. On the negative side, mistletoe can drive reduced tree growth in its hosts and result in higher forest mortality [2,3].

In many cases, however, the negative effects of mistletoe on their hosts are minor except among severely infested trees [4,5]. Furthermore, although dwarf mistletoe (*Arceuthobium*) derives much of its photosynthate from its hosts and has

Table 1. Mean $\pm$ standard error effect sizes of linear mixed-effects models testing the relationship between mistletoe abundance and host acorn production, radial growth, and survivorship, including the interactions between mistletoe abundance and species; *p*-values in parentheses. Although listed, the interactions were not significant overall by log-likelihood tests. Values for the species are relative to *Q. kelloggii*.

independent variables	dependent variable		
	acorn production	radial growth	survivorship
mistletoe abundance	0.034 ± 0.011	-0.126 ± 0.344	-0.067 ± 0.055
	(0.003)	(0.71)	(0.23)
DBH	0.005 ± 0.001	-0.001 ± 0.011	0.012 ± 0.002
	(<0.001)	(0.90)	(<0.001)
Q. lobata	0.297 ± 0.114	-1.284 ± 1.121	-1.005 ± 0.456
	(0.009)	(0.25)	(0.03)
Q. douglasii	0.277 ± 0.115	-2.604 ± 1.149	-1.902 ± 0.568
	(0.02)	(0.02)	(0.001)
mistletoe abundance $\times$ *Q. lobata*	-0.003 ± 0.031	0.547 ± 0.583	-0.466 ± 0.67
	(0.92)	(0.35)	(0.49)
mistletoe abundance $\times$ *Q. douglasii*	-0.052 ± 0.022	0.105 ± 0.415	-0.404 ± 1.042
	(0.02)	(0.80)	(0.70)

Table 2. Mean $\pm$ standard error effect sizes of general linear models testing the effects of ecological variables on the presence/absence of mistletoe including the interactions between the focal independent variable and species; *p*-values in parentheses. Although listed, interactions between the dependent variable and 'species' were not significant overall (after Tukey's correction in the case of soil *N*). Values for the species are relative to *Q. kelloggii*.

independent variables	focal independent variable (FIV)			
	mean DBH (cm)	mean xylem water potential (MPa)	mean soil N (mg l^{-1} effluent)	mean soil P (mg l^{-1} effluent)
FIV	0.015 ± 0.005	0.597 ± 1.211	0.088 ± 0.047	0.019 ± 0.027
	(0.006)	(0.62)	(0.06)	(0.48)
Q. lobata	-0.382 ± 0.604	-1.882 ± 1.991	1.626 ± 1.294	-0.013 ± 1.462
	(0.53)	(0.35)	(0.21)	(0.99)
Q. douglasii	-0.001 ± 0.645	-0.105 ± 2.125	1.216 ± 1.292	0.202 ± 1.531
	(0.99)	(0.96)	(0.35)	(0.90)
FIV $\times$ *Q. lobata*	-0.010 ± 0.007	-1.159 ± 1.474	-0.094 ± 0.048	-0.010 ± 0.031
	(0.17)	(0.43)	(0.05)	(0.75)
FIV $\times$ *Q. douglasii*	-0.012 ± 0.009	0.261 ± 1.393	-0.077 ± 0.047	-0.013 ± 0.036
	(0.20)	(0.85)	(0.11)	(0.69)

significant detrimental effects on tree growth and mortality, *P. villosum*, studied here, is a member of a primarily autotrophic genus dependent on water and minerals obtained from the xylem rather than nutrients derived from the phloem of its hosts [1,9].

It is consequently not surprising, particularly given the generally moderate degree to which trees harboured mistletoe in this study, that we failed to detect significant negative effects of *Phoradendron* presence on its hosts. More surprising is the overall *positive* relationship we found between mistletoe and acorn production. This finding is particularly unexpected given the importance of water limitation in the Mediterranean climate of our study sites [22].

A potential explanation for this result is relationships among mistletoe, avian dispersers and the ecological factors included in this study. Mistletoe was somewhat more likely to survive and grow on larger trees growing in nitrogen-rich soils, particularly in the case of *Q. kelloggii*, which generally harboured more mistletoe than the other two species (electronic supplementary material, figure S4). Once present, mistletoe would drive positive feedbacks as it produced litter and attracted species—particularly birds—to its fruit and foliage, resulting in fertilizing effects such as have been well documented in other systems [23]. Alternatively, some mistletoe-dispersing birds may prefer trees that produce more acorns, resulting in the correlation between acorn production and mistletoe. In either case, mistletoe would end up being found more frequently on healthy, productive individuals and thus be indicative of good health rather than a sign of decline.

Mistletoe's positive effects on biodiversity and the importance of its berry crop for animals provide strong incentives for exercising caution when it comes to removal of mistletoe for forest management. Except in cases of severe infestation, the negative effects of mistletoe may be small or, as found here, negligible. Indeed, *P. villosum* may reasonably be considered commensal, resembling an epiphyte more than a parasite [24]. Mistletoe can even be indicative of healthy trees with relatively high productivity, at least in terms of seed production. In such cases, the positive effects of mistletoe clearly outweigh the relatively minor negative effects it may have on its host, justifying mistletoe's designation as a keystone ecosystem resource [21].

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