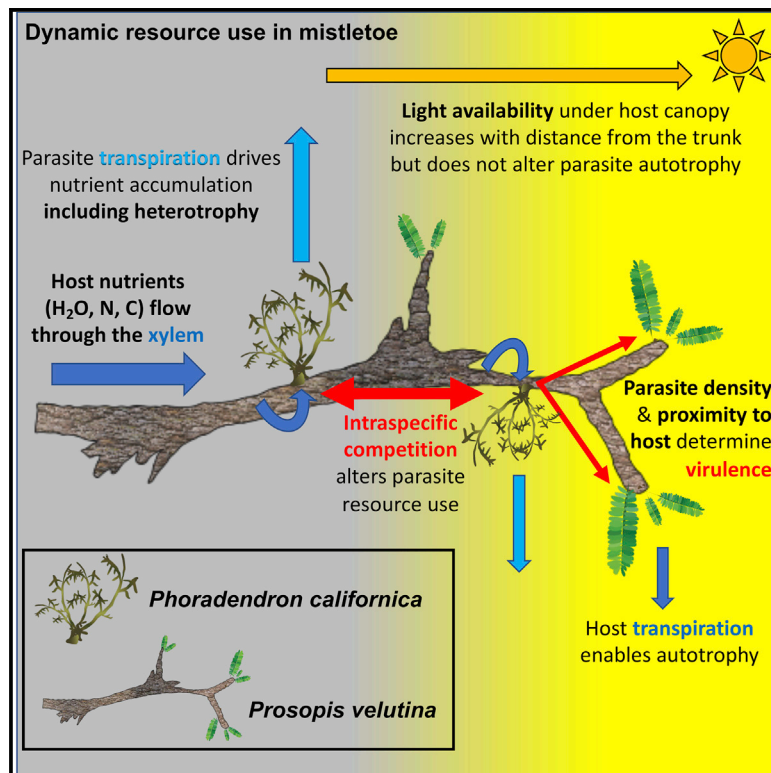


Current Biology

Intraspecific competition for host resources in a parasite

Graphical Abstract



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In Brief

Nabity et al. experimentally manipulate the resource demand in a plant parasite to show evidence for intraspecific competition. Location within a host determines both the strength of competition and virulence. Here, co-infections attenuate virulence to maintain access to host resources in a stressful environment.

Highlights

- Intraspecific competition for xylem resources exists between mistletoe individuals
- Environmental context alters the strength of competition and virulence
- Trophic flexibility tempered intraspecific competition for resources
- Co-infections attenuate virulence to maintain access to host resources



Report

Intraspecific competition for host resources in a parasite

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<https://doi.org/10.1016/j.cub.2021.01.034>**SUMMARY**

Intraspecific competition among parasites should, in theory, increase virulence, but we lack clear evidence of this from nature.^{1–3} Parasitic plants, which are sessile and acquire carbon-based resources through both autotrophy (photosynthesis) and heterotrophy (obtaining carbon from the host), provide a unique opportunity to experimentally study the role of intraspecific competition for nutrients in shaping the biology of both parasite and host.^{4–6} Here, we manipulated the spatial position of naturally occurring individuals of desert mistletoe (*Phoradendron californicum*), a xylem hemiparasite, by removing parasites from co-infected branches of a common nitrogen-fixing host, velvet mesquite (*Prosopis velutina*), in the Sonoran Desert. We measured physiological performance of both host and parasite individuals under differing competitive environments—parasite location along the xylem stream—through time. Performance was determined by measuring resource availability and use, given that resource demand changed with competitor removal and monsoon-driven amelioration of seasonal drought. Our principal finding was that intraspecific competition exists for xylem resources between mistletoe individuals, including host carbon. Host performance and seasonal climate variation altered the strength of competition and virulence. Hemiparasitic desert mistletoes demonstrated high heterotrophy, yet experimental removals revealed density- and location-dependent effects on the host through feedbacks that reduced mistletoe autotrophy and improved resource availability for the remaining mistletoe individual. Trophic flexibility tempered intraspecific competition for resources and reduced virulence. Mistletoe co-infections might therefore attenuate virulence to maintain access to resources in particularly stressful ecological environments. In summary, experimental field manipulations revealed evidence for intraspecific competition in a parasite species.

RESULTS

Is there intraspecific competition among parasite individuals on the same host branch? Ecological theory predicts that resource limitations define the competitive environment among organisms. Nutrient supply, for example, strongly influences infection dynamics and interactions within and among parasite species.^{7,8} Specifically, within-host competition among parasite strains for host resources (co-infections) should lead to the evolution of increased virulence.⁷ Although most studies addressing competition have focused on animal hosts,⁹ sessile, parasitic plants present several advantages for studying how physiological processes modulate individual parasite performance and virulence on the host. Here, we measured the extent of intraspecific competition in desert mistletoe (*Phoradendron californicum*) co-infections within velvet mesquite (*Prosopis velutina*) hosts.

We found evidence for intraspecific competition for carbon and water through a mistletoe-removal experiment. One week after mistletoe removal, conductance and transpiration decreased in proximal and distal removal treatment groups

compared with the mistletoe experiencing no removal and relative to pre-removal conditions (Figure 1; Table 1). In contrast, mistletoes experiencing no removal on their host branch did not change in conductance or transpiration but did increase photosynthesis compared to pre-removal conditions. Total nitrogen decreased for all mistletoes regardless of treatment, but total C only decreased in mistletoes experiencing competitor removal. As a result, C/N ratios increased to values similar among all treatments (Figure 1; Tables 1 and S1).

Mistletoe responses to removals depended on whether the individual removed was upstream or downstream of the individual remaining, as predicted. Conductance in distal individuals remaining downstream declined to 40% less than pre-removal levels ($t_{42} = -3.614$; $p = 0.001$), whereas proximal individuals remaining upstream did not change conductance ($t_{43} = -1.384$; $p = 0.17$) (Figure 2; Table S1), and photosynthesis did not change for either. Transpiration decreased in both distal and proximal individuals remaining after removal ($t_{42} = -3.614$, $p = 0.001$; $t_{42} = -2.642$, $p = 0.013$) (Figure 2; Table S1). In the no-removal treatment, mistletoe photosynthesis rate increased (~50%;



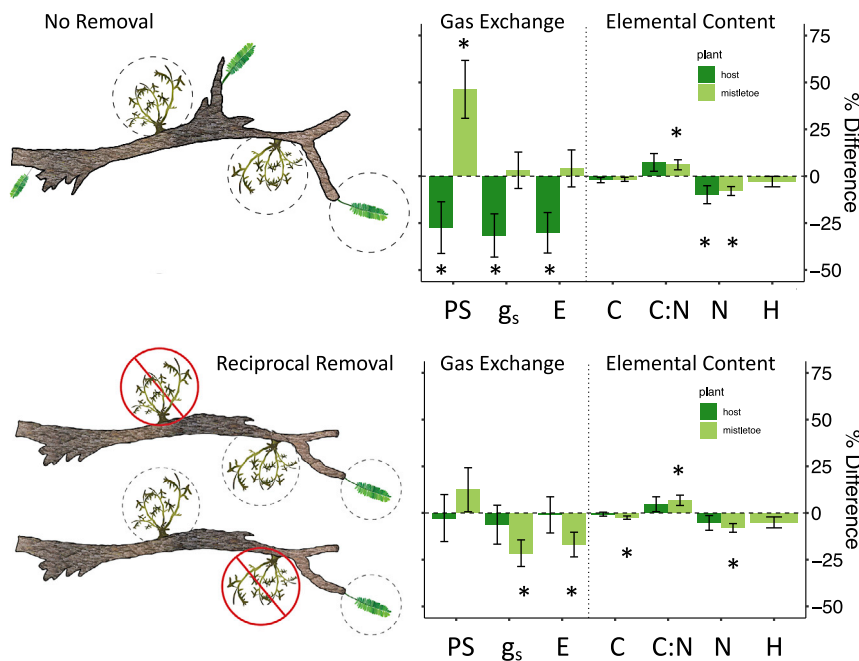


Figure 1. Change in overall performance after mistletoe removal

Experimental design (left) and mean (\pm standard error) percent changes (right) in performance and resource availability for both host and mistletoe 1 week after experimental removal. Dotted circles indicate the sites of measurement. Under reciprocal removal (bottom panel), only distal (top branch) and proximal (bottom branch) mistletoe individuals remained. Photosynthesis (PS), CO_2 conductance (g_s), transpiration (E), total carbon (C), carbon:nitrogen ratio (C:N), total nitrogen (N), and heterotrophic carbon gain (H) were measured. Differences ($p < 0.05$) from zero are denoted with an asterisk. See also [Figures S1](#) and [S2](#).

$t_{43} = 2.885$; $p = 0.006$) in proximal individuals, but not in distal individuals ($t_{43} = 1.502$; $p = 0.14$). However, conductance, transpiration, and light availability did not change compared with pre-removal conditions ([Figures 2](#) and [S1](#); [Table S1](#)). Total N declined for all mistletoe, whereas total C declined for only distal mistletoe under removal but did not change for other mistletoe ([Figure 2](#); [Table S1](#)). These changes in elemental composition resulted in increased C:N values for the proximal mistletoe with adjacent mistletoe removed and distal mistletoe with no neighbor removed. Thus, under competition, proximal parasite individuals maintained a lower C:N optimum than distal individuals on the same branch ($t_{41} = -2.046$; $p = 0.048$) ([Tables 1](#) and [S1](#)).

With the onset of the monsoon season (September), removal-induced variation in gas exchange among mistletoes dissipated, and all mistletoe increased photosynthesis and gas exchange rates. This reduced heterotrophy among all mistletoes except proximal individuals in removal treatments, although both N and C content continued to decline ([Figure S1](#)). As conditions became cooler and more arid in winter (November), photosynthesis remained similar among mistletoe, whereas conductance and transpiration decreased for all mistletoes compared with pre-removal conditions ([Figure S1](#)).

Does host quality vary spatially, and does this influence parasite performance?

Host quality influences parasite success.^{2,3,10} For plant parasites in arid climates, host vigor depends on the spatial environment, with proximity to roads and to litter fall from a neighbor altering resource availability.^{11–13} Therefore, we tested for covariance of mesquite and mistletoe traits in relation to host size and proximity to resources determined by an adjacent road and the nearest neighbor. In general, mesquite host gas exchange and elemental content did not vary with these potential drivers of nutrient availability. However, distance to the adjacent road

negatively correlated with transpiration rate throughout the seasons (E; $t_{137} = -2.363$; $p = 0.0272$). In contrast, nitrogen isotopic composition positively correlated with road ($\delta^{15}\text{N}$; $t_{137} = 4.367$; $p = 0.0001$), whereas carbon isotopes, i.e., a strong proxy for water use efficiency (WUE), negatively correlated with distance to neighbor ($\delta^{13}\text{C}$; $t_{137} = -2.252$; $p = 0.029$) across seasons. Upon parasite removal in spring, as temperatures increased and water availability decreased, the distance to road became more important, as it tended to covary with treatment-induced changes in gas exchange ([Tables 1](#) and [2](#)). However, changes in host N induced by parasite removal did covary with both distance to road and to nearest neighbor, indicating host N fixation depended on the density of parasites and resource availability to the host ([Tables 2](#) and [S2](#)).

Across the seasons, neither host size, distance to road, nor distance to neighbor covaried with any mistletoe gas exchange variables but elemental isotopes, and the calculation of heterotrophy differentially covaried with host size ($\delta^{13}\text{C}$: $t_{201} = -2.573$; $p = 0.011$), road ($\delta^{15}\text{N}$: $t_{201} = 6.609$, $p < 0.001$; $\delta^{13}\text{C}$: $t_{201} = 2.230$, $p = 0.03$; H: $t_{195} = 2.681$, $p = 0.013$), and neighbor ($\delta^{13}\text{C}$: $t_{201} = 2.3644$, $p = 0.02$; H: $t_{195} = 4.158$, $p < 0.001$). Similar to host isotopic signature, nitrogen isotopes positively correlated with distance to road ($\delta^{15}\text{N}$: $t_{196} = 6.609$; $p < 0.001$). Mistletoe carbon isotopes negatively correlated with host size ($\delta^{13}\text{C}$: $t_{196} = -2.573$; $p = 0.011$) but positively correlated with proximity to road ($\delta^{13}\text{C}$: $t_{196} = 2.23$; $p = 0.028$) and to neighbor ($\delta^{13}\text{C}$: $t_{196} = 2.364$; $p = 0.02$) across the seasons. As a result, estimates of heterotrophy also positively correlated with distance to road (H: $t_{196} = 2.681$; $p = 0.012$) and neighbor (H: $t_{196} = 4.158$; $p < 0.001$). Together, these patterns indicate host water use and efficiency depend on spatial environmental patterns, including water runoff and potential competition from adjacent individuals, and that these changes to host performance alter parasite performance.

Is there density-dependent virulence?

The degree of relatedness between parasite individuals is predicted to play a strong role in the evolution of virulence.¹⁴ Kin selection should generally favor reduced competition between parasite strains, which in turn should dampen virulence.¹⁵

Table 1. Mistletoe response after competitor removal: is there intraspecific competition?

Resource	Removal: yes	Removal: no	Difference (y-n)		Significant	Mistletoe response
	Mean (SE)	Mean (SE)	t	p	Covariates	
C: heterotrophic (H)	-0.04 (0.02)	-0.03 (0.02)	-0.372	0.71	canopy ^a	heterotrophy depends on host size
C: PS ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	0.18 (0.18)	0.52 (0.17) ^a	-1.551	0.132	Not Significant (NS)	PS increased under competition
C: g_s ($\text{mol CO}_2 \text{m}^{-2}\text{s}^{-1}$)	-0.0121 (0.004) ^a	0.0013 (0.003)	-3.013	0.005	NS	CO ₂ uptake decreased with removal
C: total percent	-1.23 (0.42) ^a	-0.86 (0.45)	-0.94	0.35	NS	no change in C with removal
C:N	0.87 (0.39) ^a	0.84 (0.31) ^a	-0.386	0.702	NS	seasonal increase in C:N
N: total percent	-0.28 (0.08) ^a	-0.30 (0.07) ^a	0.345	0.732	NS	seasonal decrease in N
Water: E ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	-0.44 (0.18) ^a	0.08 (0.16)	-2.492	0.0163	road ^b	water uptake declined with removal and depended on distance from road

Change in resource conditions 1 week after removal for desert mistletoe. Differences in resources in relation to treatment are shown with test statistics (t and p) and covariates identified as significant and trending. Photosynthesis (PS), CO₂ conductance (g_s), transpiration (E), total carbon (C), carbon:nitrogen ratio (C:N), total nitrogen (N), and heterotrophic carbon gain (H) were measured. See also [Figure S1](#) and [Table S1](#).

^aSignificant covariates ($p < 0.05$). Resources that differed from the previous (pre-removal) time point (thus $>$ or $<$ 0).

^bTrending covariates ($p < 0.1$)

Relatedness also influences plant-plant interactions, leading to various forms of cooperation with direct effects on individual performance.^{16,17} Desert mistletoe individuals within a host are more closely related than expected.¹⁸ Using this conceptual framework, we next dissected the extent to which two mistletoes on the same branch competed for host resources and how this affected host performance. When two mistletoes occurred on the same branch (co-infection), photosynthesis rate, conductance rate, transpiration rate, and N availability declined in hosts as spring advanced (May-pre-removal to May-post-removal) ([Figure 1](#); [Table 2](#)). In contrast, after at least 1 week, removal of one of the two mistletoe individuals ameliorated any declines in host performance or resource availability. The effects of parasite removal on the host dissipated during the summer monsoon season and as cooler conditions returned during fall ([Figure S1](#)). This indicates that the impact on host fitness likely depends on host stress arising from seasonality (water availability and air temperature). The proximity of host leaves to the parasite also determined how the host responded after parasite removal. In hosts where the distal parasite was removed, the host did not alter performance. When the proximal parasite was removed, host decreased conductance but only tended to lower transpiration rate ($t_{31} = -1.842$; $p = 0.076$) and did not alter photosynthesis ([Figure 2](#); [Table S2](#)). This suggests that mistletoe effects on host performance (i.e., virulence) increase with proximity to host sites of photosynthesis.

How does light availability influence mistletoe-mesquite interactions?

Stem hemiparasites are well adapted to low-light conditions and function similarly to understory plants shaded by a closed canopy. Thus, the majority of light and C gain might accrue from temporary gaps in the canopy (i.e., sunflecks)^{19,20} and maximizing photosynthesis at low light intensity.²¹ Thus, mistletoes might encounter enough light within the canopy where daily

levels often exceed $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ to provide the minimal autotrophic gains necessary for growth, especially when heterotrophy is so high. This would allow for physiological plasticity in mistletoe photosynthesis in relation to host resources (i.e., taking advantage of light when available) and buffer metabolism when N becomes limiting.

Light availability did not alter mistletoe performance. Prior to host leaf flush (in April), upstream (basal) mistletoes grew in more shade than downstream (distal) mistletoes (downstream; $t_{42} = 2.235$; $p = 0.031$; [Figure S2](#)). Shade increased for all mistletoes as host leaves flushed, but mistletoe photosynthesis increased. Upstream mistletoes still received more shade than downstream mistletoe individuals ($t_{68} = 2.244$; $p = 0.028$), and this difference remained throughout the season ([Figure S2](#)). However, mistletoe photosynthesis did not differ between mistletoe locations on the xylem stream. Prior to leaf flush in April, upstream mistletoes had higher conductance (g_s : $t_{40} = 2.765$; $p = 0.009$) and nearly higher transpiration rates (E: $t_{40} = 1.952$; $p = 0.059$) than downstream mistletoes, resulting in equal levels of both instantaneous (iWUE = Ps/E) and intrinsic (WUE = Ps/g_s) WUE between locations. These differences disappeared after leaf flush. For all mistletoes, host branch diameter was, not surprisingly, larger for upstream individuals than downstream individuals ($t_{43} = 4.718$; $p < 0.001$), providing greater xylem cross-sectional area and potential access to host resources. This resulted in greater N uptake ($t_{66} = 2.212$, $p = 0.03$; $t_{200} = 2.869$, $p = 0.005$) and a lower C:N ratio ($t_{66} = -2.151$, $p = 0.036$; $t_{200} = -2.667$, $p = 0.008$) for upstream individuals both before removal and throughout the seasons. Total C did not differ among individuals and thus was maintained at similar levels across locations. Temperature and relative vapor pressure deficit (VpdL) of mesquite and mistletoe increased ([Figure S2](#)) as gas exchange declined during spring advancement (April through May-post), but conditions were similar before and after removal in May.

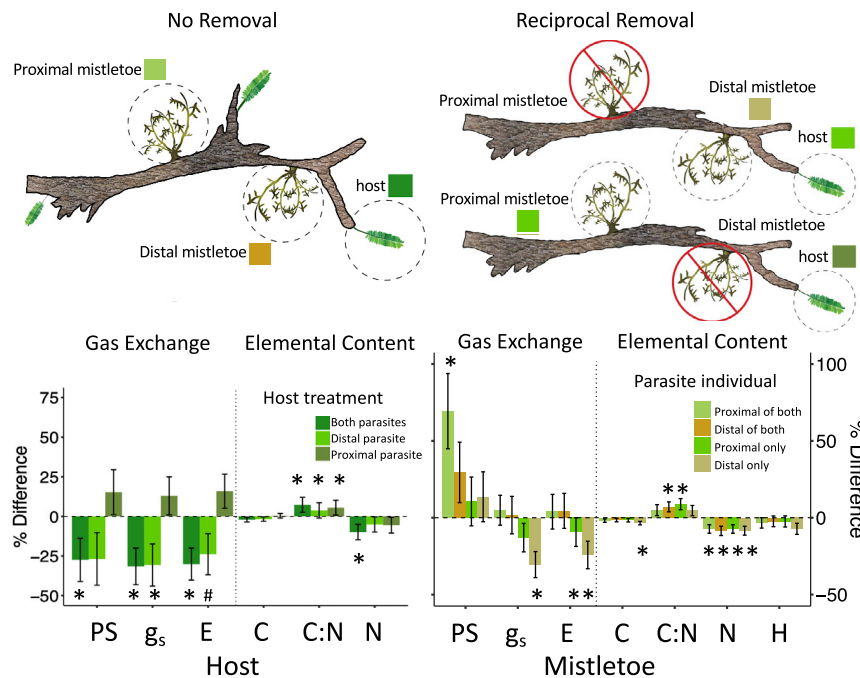


Figure 2. Change in performance of mistletoe and mesquite in relation to mistletoe location

Experimental design (top) and mean (\pm standard error) percent changes (bottom) in performance and resource availability for individual host and mistletoe 1 week after experimental removal. Dotted circles indicate the sites of measurement. Under reciprocal removal (right panel), only distal (top branch) and proximal (bottom branch) mistletoe individuals remained. Photosynthesis (PS), CO_2 conductance (g_s), transpiration (E), total carbon (C), carbon:nitrogen ratio (C:N), total nitrogen (N), and heterotrophic carbon gain (H) were measured. Differences ($p < 0.05$) from zero are denoted with an asterisk. See also Figures S1 and S2 and Table S1.

DISCUSSION

Host infection with multiple genotypes of the same parasite species (co-infection) is ubiquitous in nature. Co-infections are predicted to increase virulence because of intraspecific competition, yet there exist few experimental tests from natural systems.^{3,28} When these infections are

sublethal, feedback can occur to parasites, ultimately altering virulence.¹⁵ Here, we show that co-infection with two mistletoe individuals reduced host gas exchange compared with when one mistletoe was removed, but removal also reduced water flux through the remaining mistletoe. Because mistletoes acquire nearly all of their resources from their host plants through water transport,^{6,21,23,26} this reduction of host resources through co-infections increased virulence. Such feedback to the remaining mistletoe suggests that parasite individuals may, somehow, communicate with one another. Further, resource parasitism appears to be in equilibrium with host performance, perhaps to reduce the cost of parasitism when hot, dry abiotic conditions could jeopardize the survival of both host and parasite.

Reciprocal removal of mistletoes on the same branch also revealed location-dependent effects of parasitism. Proximity of the parasite to host leaves determined how the host responded upon parasite removal. Removal of distal mistletoes did not alter host carbon assimilation or gas exchange in adjacent leaves, but lack of removal decreased these trait values under the same abiotic context. Thus, removal might have released the host from the effects of parasitism by making more water and nutrients available. With the removal of a proximal mistletoe, the host experienced reduced conductance over time and lower photosynthesis and transpiration compared with mistletoe remaining in the proximal location. In this case, a parasite remaining closer to the site of measurement reduced host performance but alleviated the reduction in N that occurred with no removal. Removal of the individual with access to the most resources (greatest cross-sectional area of xylem) increased water and nutrient availability to the host (both C and N levels stabilized) and began to improve host quality. Similarly, distal mistletoes reduced their water and nutrient transport after removal of a proximal mistletoe. But distal mistletoes with a proximal

Do desert mistletoe and velvet mesquite perform as expected under arid conditions?

As parasites, mistletoes can reduce host fitness by transpiring host xylem-derived water at high levels in relation to carbon assimilation.^{6,22,23} Yet, remarkably, xylem-tapping mistletoes are also heterotrophic and can obtain most of their assimilated carbon from the host.^{24–26}

WUE and its relationship to plant N status varied with season but largely followed expectations. Both mesquites and mistletoes showed strong positive relationships between photosynthesis and transpiration (mesquites: $t_{136} = 18.901$, $p < 0.0001$, $R^2 = 0.85$; mistletoes: $t_{254} = 5.643$, $p < 0.0001$, $R^2 = 0.60$; Figure S2), and the slope for mistletoes is lower, as expected given its parasitic nature.⁶ Mesquites also showed no relationship between WUE and N_{area} ($t_{136} = -0.47$; $p > 0.6$), although there was an effect of time of year on WUE when increased water availability during the monsoon season resulted in a negative relationship (September $t_{136} = -3.601$; $p = 0.0004$; $R^2 = 0.22$; Figure S2). Thus, for parasitized host plants capable of N fixation, there was no enhancement of WUE in relation to N_{area} and a seasonal decline when water availability increased for hosts. Similarly, mesquite showed a negative relationship between photosynthesis and N_{area} (N_{area} : $t_{136} = -2.109$; $p = 0.037$). No relationship was found between conductance and N_{area} ($t_{136} = -0.195$; $p > 0.8$), in line with expectations that N enhancement of carbon assimilation does not occur for water-limited, N_2 fixers in arid climates.²⁷ Mistletoes partially mirrored these patterns with no relationship between photosynthesis and N_{area} ($t_{199} = 0.676$; $p = 0.5$) but showed a positive relationship between conductance and N_{area} ($t_{199} = 2.458$; $p = 0.0148$), ultimately resulting in no relationship between WUE and N_{area} ($t_{199} = -1.072$; $p = 0.29$).

Table 2. Host response after mistletoe removal: is there density-dependent virulence?

Resource	Removal: yes	Removal: no	Difference (y-n)		Significant	Host response
	Mean (SE)	Mean (SE)	t	p	Covariates	
C: PS ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	-0.12 (0.81)	-1.93 (0.67) ^a	2.017	0.0564	road ^b	PS decreased with greater parasite density
C: g_s ($\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	-0.002 (0.005)	-0.01645 (0.004) ^a	2.12	0.045	road ^b	CO ₂ uptake decreased with greater parasite density
C: total percent	-0.314 (0.47)	-1.053 (0.79)	1.2	0.24	road ^b	no change in C storage
C:N	0.72 (0.55) ^a	1.13 (0.69) ^a	-0.95	0.35	neighbor ^a	seasonal increase in C:N covaried with distance to neighbor
N: total percent	-0.175 (0.13)	-0.330 (0.13) ^a	1.474	0.15	neighbor ^a road ^a	greater parasite density decreased N, but this varied with distance to both road and neighbor
Water: E ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	-0.01 (0.22)	-0.75 (0.20) ^a	2.675	0.0142	NS	water flux decreased under greater parasite density

Change in resource conditions 1 week after removal for velvet mesquite hosts (B). Differences in resources in relation to treatment are shown with test statistics (t and p) and covariates identified as significant and trending. PS, g_s , E, C, C:N, N, and H were measured. See also Figure S1 and Table S1.

^aSignificant covariates ($p < 0.05$). Resources that differed from the previous (pre-removal) time point (thus $>$ or <0).

^bTrending covariates ($p < 0.1$)

competitor (no removal treatment) did not reduce transport. Altogether, this reveals that removal benefited the remaining distal mistletoe more than the host because demand for water and nutrients was decreased in the parasite. These changes suggest mistletoe density influenced mistletoe demand for water, and the transported nutrients tempered virulence on the host. In these experiments, we cannot exclude the possibility that mistletoe removal itself was perceived as wounding by the host.

Reductions in virulence occur with intraspecific competition,¹⁵ but high relatedness among parasites can also feedback to influence performance.^{14,16,17} Thus, mistletoes might have evolved to cooperate in order to maintain the host. This is expected if related individuals perform similarly because of shared genetic architecture or recognize one another through some means of communication. Because competition between mistletoe individuals became less intense through time, seasonal acclimation to resource availability might occur, reinforcing the conclusion that the abiotic environment strongly influences hemiparasite interactions.²⁹ Moreover, because these experiments were conducted in 1 year that included a drought (although this year was neither El Niño or La Niña), hosts might have been more stressed than in wetter years, and clearly, this could weaken competition.

Competition alters carbon acquisition

Studies on resource competition among hemiparasite species have largely focused on manipulating autotrophy through shading and N through fertilization and almost solely on root hemiparasites. Host shade reduces parasite biomass $>30\%$,³⁰ increases heterotrophy ($\geq 50\%$),³¹ and when combined with low water and varying N, increases C transfer from the host, despite no change in heterotrophy.³² Thus, at least in root hemiparasites, light levels determine carbon and nutrient uptake from the host. In contrast, we found light availability varied with host phenology and location within a host canopy, but mistletoe photosynthesis did not correlate with light availability. Proximal mistletoes encountered more shade than distal mistletoes, both prior to and after leaf flush in the spring. Despite this difference, photosynthesis was similar within each time point but

changed after removal of a competing mistletoe and with seasonal rains. During arid spring conditions, competition increased photosynthesis without concurrent changes in conductance or transpiration, thus improving assimilation efficiency in relation to changes in host nutrient transfer through the xylem. Changes in photosynthetic efficiency are known for other mistletoes, especially under N enhancement.³³ However, leguminous plants like velvet mesquite are able to fix nitrogen through symbioses with soil bacteria, typically maintaining a steady N supply, and do not amplify photosynthesis under N addition, as in non-legumes.²⁷ We found that hosts and mistletoes can increase photosynthesis or conductance with less N available. This indicates that (1) desert mistletoe individuals track host physiology because they lack N-driven stimulation in photosynthesis and (2) increasing N above a critical threshold might reduce the competition for it.

We found that spatial patterns of resource availability, including competition among hosts for water, altered host quality and performance. This effect on the host altered mistletoe performance. As hosts increased in distance from the road, water flux declined, but as hosts increased in distance from one another, efficiency (via $\delta^{13}\text{C}$ proxy) also declined. This suggests competition among trees might determine host water efficiency, perhaps to balance C gain when stomata open. However, mistletoe WUE declined with increasing host size but increased with distance to road and host neighbor. As hosts became less efficient in water use, mistletoes gained in WUE. These patterns suggest that mistletoe performance depends on host xylem flux and that C transfer feeds back to photosynthesis.^{33,34} The extent to which unknown signaling molecules or sink capacity provide feedback to adjacent competitors or the elevated N levels found in leguminous hosts buffer intraspecific competition or virulence compared with non-nitrogen fixing hosts are outstanding questions. It is likely that communication through the xylem by an adjacent competitor is influencing mistletoe performance, as is found in plant parasite volatile communication (mediated by transpiration),³⁵ and might explain how different mistletoe species facilitate co-infections.^{36,37}

In parasites where heterotrophic gains are greater than autotrophic carbon production, carbon availability might modulate intraspecific competition, influencing both parasite fitness and host fitness (through virulence). In plants, carbon allocation can be sink driven (determined by growth)³⁸ and solutes or water move laterally between phloem and xylem.^{39,40} Therefore, xylem-tapping parasites might encounter greater crosstalk and transfer of carbon between sources (sites of photosynthesis) and sinks than is generally appreciated. Altogether, we found that removal of stem hemiparasite individuals with access to the most host resources enhanced performance of the remaining parasite as well as the host. We also found competition tempered virulence, in part, because of increased autotrophy under limiting resources. Our removal experiment indicates that photosynthetic plasticity might buffer the competitive environment of autotrophic organisms that also rely on hosts for heterotrophic C gain. Gas exchange varied in relation to abiotic and biotic conditions that suggest stem hemiparasites balance resource acquisition, including photosynthesis by what is available in the xylem, and are less influenced by light availability. This is a departure from what is expected from typical photosynthetic organisms and root hemiparasites (e.g., Těšitel et al.³¹) and is consistent with sink-driven modifications of photosynthesis.³⁸

In summary, we provide experimental evidence of intraspecific competition for host resources in natural field conditions between genotypes of a parasite species. Given the ubiquity of co-infections across the diversity of parasites, mistletoes are well suited to answer classic questions that have been inaccessible in parasites, including whether kin recognition attenuates virulence in co-infections. Finally, our data provide empirical support for tradeoffs between heterotrophy and autotrophy when resource availability shapes species interactions. These results suggest that mistletoes might also prove a useful physiological model for understanding how sink-source dynamics function ecologically and ultimately evolved.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at Mendeley Data: <https://doi.org/10.1016/j.cub.2021.01.034>.

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AUTHOR CONTRIBUTIONS

P.D.N. led and conceived the study, conducted the field research, analyzed the data, and drafted the manuscript. N.K.W. helped conceive the study and provided resources. G.A.B.-G. provided resources and equipment. All authors discussed the results and edited the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and analyzed data	This Paper; Mendeley Data: https://data.mendeley.com/	https://doi.org/10.17632/v9cw7xzvkt.1
Software and algorithms		
ImageJ	https://imagej.nih.gov/ij/	https://imagej.nih.gov/ij/
R	https://www.r-project.org	https://www.r-project.org

RESOURCE AVAILABILITY

Lead contact and materials availability

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Paul Nabity (pauln@ucr.edu). This study did not generate any new reagents.

Data and code availability

Original data have been deposited to Mendeley Data: <https://data.mendeley.com/> <https://doi.org/10.17632/v9cw7xzvkt.1>

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Theory predicts that resource limitations define the competitive environment among organisms, and influences infection dynamics among parasite populations.^{7,8} Similarly, the influence of parasites extends well beyond effects on individual host performance to modulating ecosystem processes, energy flow, trophic dynamics, and buffering how hosts respond to climate change.^{29,37,41–43} Among plant parasites, the mistletoe clade within the Santalales includes keystone species that influence ecosystem diversity⁴³ and typically form parasitic connections (haustoria) on stems and branches of plant hosts where they directly tap into the host vascular system.⁴⁴ The North American genera *Arceuthobium* and *Phoradendron* colonized North American deserts from the central Mexican highlands, where climatic conditions range from arid to semiarid.^{4,5} Parasitic plants span a continuum from complete (heterotrophic holoparasites) to partial dependence (heterotrophic and autotrophic hemiparasites) on their hosts for resources. For hemiparasite individuals, resource limitation can be attenuated by autotrophy, potentially reducing competition and promoting co-infection.

Parasitism evolved multiple times across seed plants: ca. 1% of angiosperms deriving some nutrients from another plant individual.⁴⁵ Indeed, one hypothesis is that parasitism evolved in mistletoes as a means to acquire both water and nutrients.⁶ This suggests resource limitations within the host may alter performance of individual mistletoes, resulting in within-host competition and, potentially, higher virulence. Two strategies of parasitism exist among North American mistletoes that are defined by their nature of host connectivity (phloem versus xylem) and autotrophic performance.^{21,22,24,46} These strategies have varying effects on host physiology but one shared response is significant manipulation of water flow through altered host architecture⁴⁷ and elevated transpiration rates.^{48,49} Yet, no experimental manipulations have directly evaluated intraspecific resource competition between parasites on the same host (co-infections), or its potential impacts on performance of both parasite and host. Doing so may reveal how mistletoe facilitate coexistence both within populations and among species³⁶ and the potential for kin selection in attenuation of virulence within hosts.

All of these features indicate mistletoe-host interactions represent a model system to examine the mechanisms underlying intra-specific competition in parasites and implications for parasite and host fitness.

METHOD DETAILS

Experimental setup

We studied intraspecific competition in desert mistletoe (*Phoradendron californicum*) co-infections in velvet mesquite (*Prosopis velutina*) hosts. We then measured ecophysiological and elemental traits of parasites and hosts before and after performing a reciprocal removal experiment of mistletoe occupying the same branch. *Phoradendron* species acquire limited carbon (C) through photosynthesis, and generally maintain high heterotrophy.³⁴ Host carbon availability may therefore be a limiting resource for mistletoes, resulting in intraspecific competition for resources, especially when individuals are found on the same host branch and in the same xylem

stream. We predicted that upon removal from a host branch: 1) distal mistletoes remaining downstream (with respect to xylem) would increase in nitrogen (N) and C uptake (heterotrophy; H) from the host, while reducing gas exchange because xylem transport would no longer be shared with another mistletoe in the same xylem stream, and 2) proximal mistletoes remaining upstream (with respect to xylem) would reduce gas exchange because of improved water status from loss of sinks that normally increase xylem water tension. Finally, lack of removal (controls) would reduce N and H and increase gas exchange relative to the removal treatment.

The data come from experimental manipulations and surveys conducted in the Santa Rita Experimental Range located 45 km south of Tucson, Arizona, United States (31.821°N, 110.866°W, 1120 m above sea level). The landscape is predominately a semi-desert grassland that is being converted through succession and fire suppression to savanna with woody shrubs, including velvet mesquite.⁵⁰ Average precipitation is 380 mm and accumulates in two bursts, late summer monsoons and winter rains. Most of the growing season occurs in July through September during the monsoon season.

Velvet mesquite was surveyed for presence and density of desert mistletoe along a transect roughly following Madera Canyon road (beginning: 31.83371002°N, 110.940816°W; ending: 31.82880501°N, 110.933353°W). Mesquite individuals with \geq two desert mistletoe individuals per tree were used in the experiment, but we chose trees in which the total number of parasites per tree (co-infection) was low (< 10 observed individuals) to avoid density effects on overall mesquite health. Mistletoe are aggregated in space, where most hosts lack mistletoe and few hosts support many, often relative to elevation and along resource gradients that link to behaviors of dispersal mutualists.^{51,52} Therefore, mesquite trees of similar height and location along the road were assigned treatments to form a block, reducing host and soil effects on performance. Mesquite leaves are complex where the leaf is comprised of 1–2 pairs of pinnae that contain ~10–30 miniscule (< 1 cm in length) leaflets. One pinna from the first fully expanded pinnae at the branch tip and actively growing stem tips (determined by brighter green coloration) at the vegetative stage from each mistletoe individual (which are leaf-less) were measured for gas exchange using an automated infrared gas analysis system (LI-6400 Photosynthesis System, LI-COR Biosciences, Lincoln, NE). Treatments of: 1) no removal, 2) removal of mistletoe located upstream (proximal), and 3) removal of mistletoe located downstream (distal) were randomly assigned to each host tree within a block. Mistletoes were removed from a single branch using shears or remained unaltered to serve as controls. Although removal may alter physiology downstream of the wounding site, we assessed all plant response prior to and after wounding to account for treatment application effects. Twelve blocks (36 trees) were followed through time, and no mistletoe regrowth was observed for experimentally removed plants during the experiment. Plants were surveyed in 2012–2014 with experimental removals beginning in spring 2013, which coincided with neither El Niño or La Niña climatic shifts. However, throughout the experiment the site experienced drought ranging from extreme in spring 2013 to moderate after monsoon season (<https://droughtmonitor.unl.edu>).

Plant Traits Measured

CO₂ and water use in host and parasite were measured prior to leaf flush (April), prior to mistletoe removal when host leaves were present (May-pre removal), seven days after removal (May-post removal), mid to late monsoon season (September), and prior to leaf senescence (November). For gas exchange, survey measurements were recorded every 10 s for 30 s after steady state conditions were observed using the standard 6 cm² chamber with an external light source (PAR = 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Environmental conditions inside the chamber were held constant within blocks, but temperature often varied from morning to afternoon blocks and between days depending on the ambient temperature.

To standardize measured values to leaf surface area, host tissues were collected after field surveys, maintained on ice during transport, and photographed in the laboratory over a standardized grid. Photographs were then assessed using imageJ (<https://imagej.nih.gov/ij/>) to convert pixel number within the masked leaf image to area. Because desert mistletoes are leaf-less, we calculated the surface area of stems used to measure gas exchange. Total length and the width at both proximal and distal ends were used to calculate the surface area for a truncated conical cylinder ($\pi*(d1+d2)/2*l$) where d1 is the diameter of the cylinder base, d2 is the diameter of the cylinder tip, and l is the length of the cylinder. Total variance in leaf area among mesquites (0.47–0.62) and mistletoes (0.05–0.62) varied with season but remained similar between treatments.

To assess how ambient light conditions varied relative to season and location within the tree, host tree canopy cover was measured for each mistletoe individual using a concave/convex spherical crown densiometer (Forestry Suppliers, Inc, Jackson MS, USA). The diameter of the host branch at each mistletoe location was also measured using digital calipers. Only vegetative (non-reproductive) mistletoes were selected for this experiment; however, some mistletoe individuals flowered out of season by monsoon or fall time points. If flowering occurred, plant sex was recorded given the possibility for sex-dependent differences in gas exchange in *Phoradendron* species.⁵³

Upon completion of gas exchange and canopy measurements, tissues from all plants were collected immediately and temporarily stored on ice during transport, then stored at -20°C prior to processing for elemental and isotopic analyses.

To determine water use efficiency (WUE), heterotrophy (H), and elemental composition, C, N, and related isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were measured for each tissue, from each species. Thus, 72 mistletoe and 36 mesquite were sampled prior to removal, and the remaining 48 and same 36 mesquite were sampled post removal. Mesquite leaf and mistletoe stem tissue were dried at 70°C , ground to a fine powder, and analyzed using an Elemental Combustion System (model 4010, Costech Analytical Technologies, Valencia, CA) coupled to a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL). Standardization was based on acetanilide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}\text{C}$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}\text{N}$. Heterotrophy was determined from [predicted $\delta^{13}\text{C}$ of mistletoe (from gas exchange data) – known $\delta^{13}\text{C}$ of mistletoe (from isotope data)] / [predicted $\delta^{13}\text{C}$ of

mistletoe - known $\delta^{13}\text{C}$ of host].^{25,54} Predicted $\delta^{13}\text{C}$ was calculated using updated values of $\delta^{13}\text{C}$ of the atmosphere (-8.4) and the net isotopic discrimination by RuBP carboxylase ($b = 29\text{‰}/\text{OO}$) from Busch et al.⁵⁵

Because host vigor and neighbor density can influence mistletoe growth indirectly by altering physiology of the infected host,⁵⁶ and resource availability in desert ecosystems can be influenced by proximity to a road and litterfall by neighbors,^{11–13} we estimated host size and proximity to the adjacent road and nearest neighbor. Estimates were made using GPS locations of host and satellite imagery. Host size was estimated via proxy by canopy area that was calculated from the elliptical area of N-S and E-W transects bisecting each tree canopy. Distances to the road midline and nearest neighbor were measured using the distance tool in Google Earth.

QUANTIFICATION AND STATISTICAL ANALYSIS

Seasonal Advancement

All data were analyzed in R version 3.6.0 (R Core Team 2019) using the package “lme4”.⁵⁷ First, we examined how mesquite and mistletoe physiology varied over time by fitting linear mixed-effects models (LMM) with either mesquite or mistletoe gas exchange and elemental parameters (see Supplemental File: [Table S2](#) for complete list) as the response variable, and sampling time point (categorical) as the predictor. For mistletoe models, we included location on the host branch (upstream versus downstream) as a predictor. Because we were also interested in how these response variables changed within sampling time points and relative to removal treatments, we fit similar models (excluding date) with the following treatments for host: no removal (both mistletoes remaining), removal (upstream mistletoe remaining), removal (downstream mistletoe remaining); and for mistletoe: no removal (upstream individual), no removal (downstream individual), removal (upstream remaining), removal (downstream remaining). To account for repeated sampling of individual trees and temporal variation in ambient temperatures when gas exchange was measured, tree identity and time-of-day of measurements were included as random effects. To account for factors that may covary and alter host quality, we included host size, distance to neighbor, and distance to road midline as covariate predictors. Model fit was determined by comparing the log-likelihood using the package “lme4.” We report only the best-fitting models.

Water use economics

To examine water use relative to carbon gain, we fit LMMs with photosynthesis or WUE of either mesquite or mistletoe as the response variable and transpiration or N_{area} , sampling time point, and their interaction as predictors. Covariates listed above were included as predictors and tree identity was included as a random effect. Model fit was determined by comparing the log likelihood values of each model (with and without the interaction), and when a simpler model fit best, the interaction term was removed. We report only the best-fitting models.

Effects of Competition

To test how mistletoe traits changed under potential competition throughout the season we fit LMMs with different plant traits as the response variable and removal treatment (see above), sampling time, and covariates as the predictors. We included random effects for time-of-day of measurements. To examine how traits varied seasonally, we fit separate LMMs for each sampling time point. For these models, we used the differences between the pre-removal (May-pre) time point and subsequent time points ($t_n - t_0$) for each parameter as the response variable and removal treatment plus the covariates as the predictors with a random effect of time. We used a random intercept model to assess treatment effects and set 0 as the intercept to test against the null expectation that treatment had no effect (difference in response = 0) over time. When covariates were not significant, i.e., did not differ from 0, they were removed from the zero-intercept model to test if treatments changed over time when all other factors do not.⁵⁸ Post hoc comparisons among treatments were made against the expectations that: 1) mistletoe remaining downstream would increase in elemental content while reducing gas exchange, 2) mistletoe remaining upstream would reduce gas exchange, and 3) no removal would increase gas exchange and decrease elemental content in both individuals.

Effects of Parasitism

To determine how host plant traits changed in response to parasite density, we used LMMs with plant traits as the response variables and removal (yes or no) plus the covariates as the predictors. We also included time-of-day as a random effect. Both random and fixed intercept models were examined as described above.