Mistletoe generates non-trophic and trait-mediated indirect interactions through a shared host of herbivore consumers

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Abstract. Indirect interactions emerge among a wide range of herbivores sharing the same plant resource. Consumers usually belong to different trophic guilds, from folivores and sapsuckers to parasitic plants. We propose that mistletoes parasitizing pines could play a key role acting as herbivores on host pines and coming indirectly into competition with other herbivores feeding on the same host. Changes caused by mistletoes on its host have been well studied, but its effects running across trophic webs remain unrevealed. In this study, we investigate the effect of European mistletoe (*Viscum album* subsp. *austriacum*) on the host-feeding herbivores via trait-mediated indirect interactions (TMIIs) across their shared pine host (*Pinus nigra* subsp. *salzmannii*). We performed field and laboratory experiments, and analyzed the net effect of different mistletoe parasite loads on three host-phytophagous species: the sapsucker *Cinara pini* (Aphididae), the winter folivore *Thaumetopoea pityocampa* (Thaumetopoeidae), and the summer folivore *Brachyderes* sp. (Curculionidae), all being members of different functional feeding groups (FFGs). We summarize the mistletoe–host–herbivore interactions by means of a TMII, where mistletoe parasitism causes non-trophic links and detrimental indirect interactions on pine-feeding herbivores across its shared host, suggesting a worsening of host quality as food. These indirect interactions vary according to three parameters. First, the intensity has a non-proportional relation with parasite load, showing an impact threshold on highly parasitized pines. Second, the movement capacity of insect herbivores determines their response, by decreasing the abundance of herbivores with low movement ability (aphids and pine processionary caterpillars) while altering the behavior (plant selection) of more mobile herbivores (pine weevils). Finally, FFG determines the intensity of mistletoe parasitism effects, folivores being more responsive than sapsuckers. Overall, mistletoe generates non-trophic interaction linkages in the forest able to modify community structure by becoming a nexus of the entire herbivore community of the pine canopy.

Key words: aphids; folivores; hemiparasite; host pine; insect herbivores; Mediterranean forest; pine processionary; pine weevil; plant–animal interaction; sapsuckers; *Viscum album*.

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Introduction

Plants are simultaneously consumed by organisms as diverse such as insects, vertebrates, fungi, nematodes, or parasitic plants. The resulting interactions between phylogenetically disparate organisms sharing a common resource appear to

be a common phenomenon, but little known (Hochberg and Lawton 1990, Wardle et al. 2004, Bass et al. 2010). In fact, studies on interactions between invertebrate consumers of the same plant have documented both competitive and positive interactions (Denno et al. 1995, Ohgushi 2005), and the consequences of the participating

species for the population dynamics have been examined (Denno et al. 2000, Ohgushi 2005). These consumers induce plant responses (Karban and Baldwin 1997) and generate interaction linkages caused by non-trophic indirect effects, able to modify the food-web structure (Ohgushi 2005, 2008). Indirect effects can result from changes in the density of a species involved in a food web, triggering interactions known as density-mediated indirect interactions (Peacor and Werner 1997, Werner and Peacor 2003) or, alternatively, changes in the phenotypes of any species (morphological, physiological, phenological, and behavioral) involved in a trophic web, thus causing the trait-mediated indirect interactions (TMIIs; Werner and Peacor 2003, Schmitz et al. 2004, Gómez and González-Megías 2007). Despite recent advances in the knowledge of indirect interactions, there are hardly any studies on the magnitude and ecological importance of the interactions between parasitic plants as initiators and insects as receptors, that use the same plant as the intermediary, and in turn as a trophic resource (Hartley and Gange 2009; but see Bass et al. 2010 and Ewald et al. 2011).

The direct impact of parasitic plants on their hosts, unlike that of herbivores, is often disproportionately strong in relation to their abundance (Watson 2001, 2009, Hartley et al. 2015) and able to restructure the plant (Pennings and Callaway 1996, Davies et al. 1997, Hódar et al. 2018) and invertebrate community (Stevens and Hawksworth 1970, Hartley et al. 2015). Parasitic plants are also the trigger for a series of indirect interactions with many other species in the community located at different trophic levels. Parasitic plants can compete with other species that consume the host plant, such as herbivores, which represent a potentially limiting shared resource (Gómez 1994, Puustinen and Mutikainen 2001). Additionally, as parasitic plants can change the morphology and architecture of their host, they can also affect other species that use the host as a habitat (Mooney et al. 2006). This is the case of mistletoes, hemiparasitic plants able to play a prominent role in the forest canopy acting as an herbivore (Ehleringer et al. 1985, Pennings and Callaway 2002, Zuber 2004), taking up minerals and nutrients through the haustorium embedded in the host xylem (Kuijt 1977, Zuber 2004). All mistletoe-induced changes on

host species could affect other host consumers in several ways, running across the trophic web to primary (herbivores) and secondary consumers (predators and insectivorous birds). Therefore, mistletoe parasitism could be the starting point for a novel case of TMIIs, where the initiator of these indirect interactions is a plant, resembling an herbivore but with effects disproportionately strong with respect to their size (Watson 2001, Hartley et al. 2015). These indirect interactions link mistletoes with host-feeding herbivores mediated by changes in traits of its shared plant-host.

In this study, we aim to investigate the indirect effects of the European mistletoe (*Viscum album* subsp. *austriacum*) on population responses of herbivores, through mistletoe-induced changes on their shared black pine host (*Pinus nigra* subsp. *salzmannii*). Using field and laboratory manipulation experiments, we tested the hypothesis that the presence of mistletoe would influence a range of insect herbivores and that this impact would be proportional to mistletoe abundance. We focused the study site in a Mediterranean mountain (Natural Park of Sierra de Baza) in southeastern Spain, where *V. album* parasitizes mainly *P. nigra*. Our study system represents a particular case because, while many plant–parasite interactions have been studied in short-lived organisms (Bass et al. 2010, Ewald et al. 2011), here we work with two long-lived plants (>300 yr for *P. nigra*, until 40 yr for mistletoe). Furthermore, pines parasitized by mistletoe show a high rate of re-infestation (Mellado and Zamora 2016), which increases the host parasite load, leading eventually to death (Mellado and Zamora 2016). Thus, while herbivory is generally episodic, increasing in intensity over short time periods (outbreaks of defoliators), mistletoe constitutes a chronic, long-term stressor, sequestering water and macronutrients continuously from the host (Schulze et al. 1984, Ehleringer et al. 1985). In a previous study, we show how the pine host responds to different mistletoe parasite loads by altering the chemical profile of pine needles, decreasing their quality as food, and synthesizing defense compounds in moderate and highly parasitized pines (Lázaro-González et al. 2019). Thus, from the standpoint of a pine-eating arthropod, there are three potential food sources: unparasitized pines, parasitized pines, and the

mistletoe itself. We know now that the last possibility is not an option, because the arthropods feeding on mistletoe are narrow specialists (Lázaro-González et al. 2017).

Herbivore insects are more likely to respond quickly to mistletoe parasitism in relation to changes in the host, due to their short life cycles and sensitivity to food quality throughout the first instars (Hódar et al. 2002, Zalucki et al. 2002). In addition, considering that mistletoes could affect not only pine needles (Lázaro-González et al. 2019), but also different pines tissues (e.g., phloem), we used three phytophagous species as a case study, according to different functional feeding groups (FFGs): the summer sap-sucking aphid *Cinara pini* (Hemiptera: Aphididae), the winter folivore pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), and the summer folivore beetle *Brachyderes* sp. (Coleoptera: Curculionidae). These three target species have a high impact as phloem and needle consumers, respectively, being common in the pine forests of our study site (A. Lázaro-González, J. A. Hódar and R. Zamora, *unpublished data*).

To elucidate the TMIIs initiated by mistletoes, firstly we explore how a gradient of mistletoe parasitism affects the presence and abundance of the three target species on wild populations on its shared host. Because of prior evidence of mistletoe-induced chemical changes in medium and high degrees of parasitism in pines (Lázaro-González et al. 2019), we expect to find lower populations at higher levels of mistletoe loads, either by site selection (actively choosing the preferred tissue) or by differences in survival or reproduction. Secondly, we test how the parasite load determines the population responses under field and laboratory experimental conditions. We expect stronger responses on insect populations fed on pines undergoing high rather than low parasitism, according to the severity of the chemical changes (Lázaro-González et al. 2019). Finally, we asked how the FFG (sap-sucking or folivore) determines the indirect effects according to gradient of mistletoe parasitism. Because pine tissues (e.g., phloem and needles) could be differently affected by mistletoe parasitism, responses of our focal herbivore species could also differ between FFGs, since they are sapsuckers and folivores, respectively.

MATERIALS AND METHODS

Study site

Mistletoe, *Viscum album* subsp. *austriacum* (Wiesb. Vollman), is a dioecious parasitic plant native to the European region that specializes in parasitizing conifers, mainly *Pinus* species, across its distribution range (Zuber 2004). This study was conducted in the Natural Park of Sierra de Baza, Granada (southeastern Spain, 2°51′48″ W, 37°22′57″ N), representing the southernmost limit of its geographical distribution. The climate at the site is typically Mediterranean, with historic annual mean temperature of 15.5°C (CMAOT 2016) and annual mean rainfall of 495 ± 33 mm (1991–2006 period; Cortijo Narváez meteorological station, 1360 m.a.s.l.) concentrated in autumn and spring, hot and dry summers (June–September), and cold winters (December–March). Conifers are the dominant forest vegetation (43% of the total surface coverage), where black pine (*Pinus nigra* Arn. spp. s*alzmannii*) is the most main species that hosts mistletoe. The site has other pine species, such as Scots (*P. sylvestris* L.), Aleppo (*P. halepensis* Mill.), and maritime (*P. pinaster* Ait.) pine, as well as oaks (*Quercus ilex* L., 9%) and shrublands (23%; CMAOT 2008).

To characterize the wild populations of the target species, we selected 55 unparasitized black pines and 55 parasitized ones, according to these mistletoe loads: control (C), trees free of mistletoe; low (L), trees <20% of canopy occupied by mistletoe; medium (M), trees 20–50% of canopy occupy by mistletoe; and high (H), trees >50% of canopy occupy by mistletoe. Sample sizes for control, low, medium, and high were 55, 17, 27, and 11, respectively. Trees were selected along an elevational gradient (from 1300 to 1850 m.a.s.l.) on the Natural Park of Sierra de Baza, considering within-forest heterogeneity such as areas with different tree densities. Trees were randomly selected and spatially paired (one parasitized and one unparasitized). Paired trees were of similar architecture, size (diameter at breast height: 25.55 ± 1.31 cm), age (90–110 yr old; see Herrero et al. 2013), and height (6.87 ± 0.23 m). These were located 40–80 m apart to control the environmental variability (e.g., climatic factors and composition of neighboring vegetation).

The experimental study was performed at Cortijo Casimiro (Natural Park of Sierra de Baza, 1400 m.a.s.l.), in a coetaneous afforestation of *P. nigra*, where trees bear various mistletoe loads. Two field bioassays were conducted in situ, while two laboratory bioassays were performed with pine needles collected at the site. We selected 40 pines according to the aforementioned four levels of parasite loads (control, low, medium, and high; 10 trees each).

Sampling of wild populations of target species

Brachyderes sp. and *Cinara pini* were sampled on July 2013 by the beating technique, in which a heavy stick was used for 10 s to beat a branch on three randomly chosen branches per pine. All non-sessile and non-flying arthropods inhabiting pine branches, including these two species, dropped onto a cloth collector (1 m^2) under the pine branches. All curculionids and aphids were sorted, identified, and counted. Additionally, we examined all pines present on a large marked plot (~2 Ha) located at 1650 m.a.s.l. during spring 2009. The plot included 230 black pines, parasitized and unparasitized ones, where the parasite load of each pine was recorded. At this area, the population of *Thaumetopoea pityocampa* was estimated by counting the number of winter nests per tree. Since the larvae are gregarious from hatching and congregate in a silk cocoon (hereafter nest) and develop during winter, the beating technique is not a reliable method for this species, while counting winter nests is a widely used procedure in most countries in which this pest is present (Battisti et al. 2015).

All data were tested in two different ways: On the one hand, we used a binomial data of presence/absence; and on the other hand, we tested their abundance. Due to lack of our target species in many samples, we used a negative binomial error distribution for abundance data. For *Brachyderes* sp. and *C. pini* data, we used a generalized linear mixed model (GLMM) where mistletoe load (C, L, M, and H) was a fixed factor, and elevation (from 1300 to 1850 m.a.s.l.) and paired trees (from 1 to 55) were random spatial factors. *Thaumetopoea pityocampa* data were run with a generalized linear model (GLM) where mistletoe load was a fixed factor. The models were run by *glm*, *glm.nb*, *glmer*, and *glmer.nb* functions of the *stats*, *MASS,* and *lmer4*

packages, respectively. Finally, Tukey's pairwise comparisons (95% confidence level) were conducted using *lsmeans* function of *lsmeans* package. R software system version 3.4.3 (R Development Core Team 2017) was used to all statistical analyses of this work. Throughout the paper, means are expressed as ± 1SE.

Field bioassay with Cinara pini

Bioassay was conducted in situ from early July to mid-September 2012. Firstly, we selected four healthy pine branches of each focal pine and wrapped the growth of the last two years with lycra bags (totaling 160 bags, 40 for each level of mistletoe load). Afterward, the bags were sprayed with pyrethroid insecticide (Coopermatic: pure natural pyrethrins [1.67%] and piperonyl butoxide [11.10%]) and closed. The insecticide was left to work for two weeks, to kill any arthropod inside the bags. Once the activity of the insecticide totally decayed, we set two parthenogenetic females of *C. pini* (Hemiptera: Aphididae) collected on healthy, mistletoe-free black pines nearby. All bags were checked biweekly, without introduction of new aphids. In mid-September, we collected all bags and transported them to the laboratory, where all aphids per bag were counted under a magnifying glass.

Just as above, we used data to test the survival and abundance per bag, considering survival to be two or more live aphids inside a bag, and abundance the number of aphids by bag. We used a GLMM with binomial distribution for presence data, while due to high mortality, we used a GLMM with negative binomial error distribution to test the abundance. In both cases, mistletoe load (C, L, M, and H) was a fixed factor and individual tree (1–40) was a random factor.

To test for a microclimatic effect of the bags, we set three pairs (A, B, and C) of data logger (HOBO UTBI-001, TidbiT v2 Temp Logger; Onset, Bourne, Massachusetts, USA), inside and outside bags on randomly selected pines. Data loggers recorded the temperature every 30 min throughout the bioassay (72 d). We analyzed four different variables of temperature: mean, minimum, maximum, and coefficient of variation. Linear mixed models were used to test bag effects on these four variables. Bag position (inside and outside) was fixed factor, time (days) was a temporal random factor, and pair (A, B,

and C) and data logger (1–6) were spatially hierarchical random factors. Coefficient of variation was calculated by standard deviation/mean temperature of daily temperature and log-transformed to meet all model assumptions. The results show that bagging did not affect the microclimatic conditions on pine twigs. Although bagging marginally increased the maximum ($F_{1,2} = 11.35$, $P = 0.078$), mean ($F_{1,2} = 8.05$, $P = 0.105$), minimum ($F_{1,2} = 2.49$, $P = 0.342$) temperatures, and coefficient of variation ($F_{1,2} = 7.03$, $P = 0.118$), the temperature inside and outside the bags did not differ (Appendix S1: Fig. S1). Thus, any effect on survival and abundance of aphids during field bioassay can be ruled out.

Field bioassay with Thaumetopoea pityocampa

Egg batches of pine processionary moth *Thaumetopoea pityocampa* were collected in a nearby (15 km apart) pine forest with similar moth phenology than Cortijo Casimiro (Hernán Valle, Granada, 3°03′ W, 37°23′ N, 1130 m.a.s.l.) during August 2013. We fixed five egg batches on the pine needles of different apical branches of the 40 focal pines (see above). Egg batches depredated by birds or bush crickets (Hódar et al. 2013) were replaced until egg batches hatched. After the larvae hatched, we labeled the nest and removed the egg batch. In the laboratory, we analyzed the empty egg batch to record the number of larvae hatched. Nests in the field were monitored weekly, and specimens were collected once they molted to the second instar. In laboratory, we counted the number of larvae that reached the second instar.

Unhatched egg batches were removed from statistical analyses. We considered survival as all nests with at least one 2nd-instar pine processionary caterpillar, and abundance as the proportion of larvae per nest and the number of eggs that hatched per batch (live vs. hatched caterpillars). Survival was tested by running a GLMM assuming a binomial distribution data, where mistletoe load (C, L, M, and H) was a fixed factor and individual trees (1–40) constituted the random factor. Due to high caterpillars' mortality, we tested differences in abundance using a GLMM with negative binomial error distribution and the same model structure than survival.

Laboratory bioassay with Thaumetopoea pityocampa

To complement the previous bioassay under controlled conditions, we performed a laboratory bioassay with pine processionary caterpillars. In August 2012, we collected 25 egg batches in a pine forest on Cortijo Quemado, Lanjarón (Granada, 3°29′41″ W, 36°56′39″ N, 1300 m.a.s.l.). These egg batches were placed inside an incubator at laboratory, under controlled conditions of temperature (15°–22°C) and humidity (40%), until eggs were hatched. Newly emerged caterpillars were placed in groups of 20 individuals in Petri dishes, with a moist base filter paper (see Hódar et al. 2002 for a similar procedure). A total of 2960 caterpillars hatched, and 148 Petri dishes were completed (ten focal pines × four mistletoe loads × three replicates, and another incomplete replicate of seven focal pines × four mistletoe loads). Each group of caterpillars was fed on daily with fresh needles of the same focal pine, until larvae had molted to the 2nd instar, as in the previous field bioassay.

When bioassay ended, we considered survival as Petri dishes with two or more live larvae, and abundance as the number of larvae on 2nd instar by Petri dish. Once again, we tested survival and abundance separately running a GLMM with binomial and negative binomial error distribution, due to high mortality, respectively. Mistletoe load (C, L, M, and H) was fixed factor, and individual tree (1–40) and reply (1–4) were random factors.

Cafeteria test with Brachyderes sp

In August 2013, we collected 40 individuals of *Brachyderes* sp. (O. Coleoptera, Fam. Curculionidae) in a pine forest on Lanjarón (Granada, 3°30′ W, 36°57′ N, 1350 m.a.s.l.). All beetles were transported to the laboratory and placed in a plastic box inside the incubator during 24 h, under controlled condition of temperature and humidity (see above), and healthy mistletoe-free black pine needles and water ad libitum, to establish the same conditions in all individuals. Then, beetles were sorted into ten containers with four individuals each and left 24 h without food. Afterward, we placed fresh needles from four focal pines (one pine per mistletoe load: C, L, M, and H) in each container. Needles were measured before the trial

(length, in mm), ensuring a similar total needle length between mistletoe loads and between containers. Pine weevils were left to feed for 24 h. Afterward, we measured the needles again and recorded the difference between initial and final length per mistletoe loads as consumption by beetles. The trials were replicated three times, randomly sorting the beetles and focal trees per container for each replicate, and the beetles were placed together at the same container between trials, with healthy mistletoe-free black pine needles and water provided ad libitum for 24 h.

To test the effect of the mistletoe load (C, L, M, and H) on feeding preferences, we considered two different variables: nibbled needles (yes–no) and length of consumed pine needles (mm). Both variables were tested using GLMM, with binomial and negative binomial error distribution, where mistletoe load was a fixed factor, and focal pines (1–40), containers (1–10), and reply (1–3) were random factors.

RESULTS

Target species on wild population

A total of 550 aphids and 97 curculionids were found on 79 and 50 pines, respectively, from 110 total sampled pines, during beating sampling, while 220 pine processionary nests were counted on 121 from 230 total pine trees. The presence (χ^2 = 1.90, df = 3, P = 0.593; Fig. 1a) and abundance (χ^2 = 2.16, df = 3, P = 0.539; Fig. 1b) of sap-sucking $C.$ $pini$ in the wild population showed no significant differences between mistletoe loads (Appendix S2: Table S1). Conversely, folivores are affected by mistletoe parasitism, decreasing their presence ($Thaumetopoea$ $pityocampa$, χ^2 = 6.91, df = 3, P = 0.075; $Brachyderes$ sp., χ^2 = 10.17, df = 3, P = 0.017; Fig. 1c–e) and abundance ($T.$ $pityocampa$, χ^2 = 8.46, df = 3, P = 0.038, and $Brachyderes$ sp., χ^2 = 12.48, df = 3, P = 0.006; Fig. 1d–f) on pines parasitized by high and medium–high parasite loads of mistletoes, respectively (Appendix S2: Tables S2, S3).

Field bioassay with Cinara pini

A total of 123 bags out of 157 contained at least two live aphids at the end of the bioassay, totaling 1290 aphids. Multiple comparisons did not reveal any difference on survival of aphids between levels of mistletoe loads (χ^2 = 4.76, df = 3, P = 0.190; Appendix S3: Table S1, Fig. 2a), but their abundance decreased significantly from unparasitized pines to those with high parasite loads (χ^2 = 14.98, df = 3, P = 0.002; Fig. 2b), with the differences between these two levels of mistletoe loads being significant (C: 15.4 ± 3.7 individuals; H: 1.9 ± 0.3 individuals), and marginal between pines of low (L: 10.3 ± 3.0 individuals) and high parasitism (Appendix S3: Table S1).

Field bioassay with Thaumetopoea pityocampa

A total of 127 hatched egg batches were analyzed; from these, 78 bore at least one 2nd instar larvae, for a total of 4614 larvae molted to 2nd instar. Survival per nest was not significantly affected by mistletoe load (χ^2 = 3.11, df = 3, P = 0.374; Appendix S3: Table S1, Fig. 2c), but there was a slight trend in mistletoe-free pines to show a higher rate of nest survival (C: 74%, n = 34) than in parasitized pines, regardless of their parasite load (L: 59%, n = 32; M: 59%, n = 29; H: 53%, n = 32). However, the pattern was much clearer when considering abundance of caterpillars inside survivor nests (χ^2 = 91.75, df = 3, P < 0.001; Appendix S3: Table S1, Fig. 2d), reflecting a significant and progressive decline from unparasitized (44.2 ± 6.4 individuals per nest) and low parasitized pines (43.3 ± 7.1 individuals per nest), to those with medium (39.9 ± 6.9 individuals per nest) followed by high parasitism (30.5 ± 6.5 individuals per nest).

Laboratory bioassay with Thaumetopoea pityocampa

In contrast to field bioassay, the results in laboratory bioassay showed clear-cut differences on survival (χ^2 = 8.99, df = 3, P = 0.029; Fig. 2e) and abundance (χ^2 = 15.62, df = 3, P = 0.001; Fig. 2f) of caterpillars between unparasitized pines and parasitized ones (Appendix S3: Table S2). Thus, survival and abundance on Petri dishes of caterpillars fed on pine needles of control (C: 32%, 4.1 ± 1.1 individuals, n = 37) or low mistletoe load pines (L: 49%, 6.3 ± 1.3 individuals, n = 37) significantly differed in survival and abundance of caterpillars fed on needles of pines with medium (M: 5%, 0.8 ± 0.6
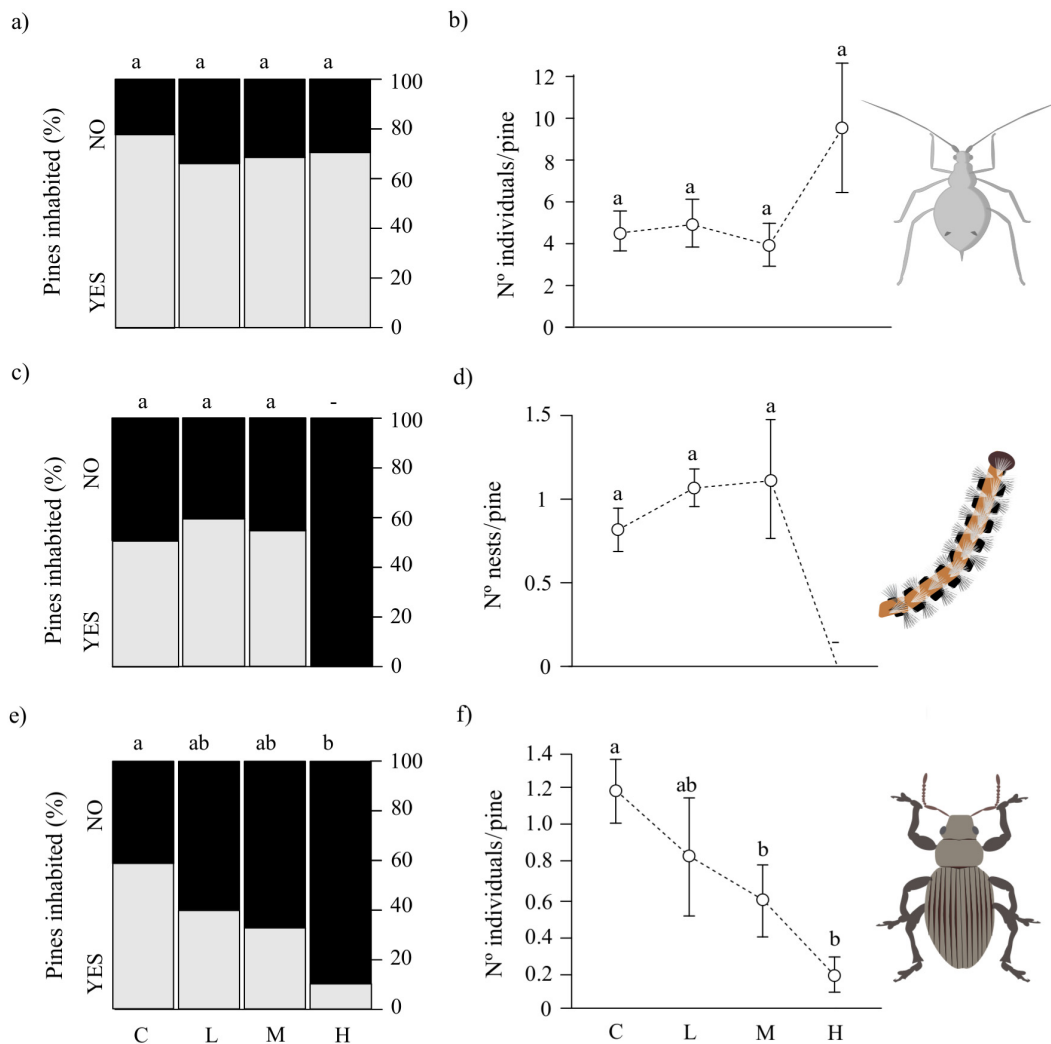
Fig. 1. Pine-feeding herbivores in natural conditions: presence/absence (left column, gray and black bars, respectively) and abundance (right column, mean ± SE) of (a, b) aphids (n = 110), (c, d) nests of pine processionary moth (n = 230), and (e, f) pine weevils (n = 110) per pine according to the mistletoe loads on the pine host (C = control, L = low, M = medium, and H = high). Differences among parasitism levels are indicated by different lower-case letters.

individuals, n = 37) and high parasitism (H: any alive caterpillar, n = 37).

Cafeteria test with Brachyderes *sp*

Although the cafeteria test with the curculionid beetles showed no significant differences in the nibbled needle (χ^2 = 5.76, df = 3, P = 0.124; Appendix S3: Table S2), samples from control (93%, n = 30) and low (97%, n = 30) mistletoe loads were more nibbled than medium (83%, n = 30) and highly (77%, n = 30) parasitized pines (Fig. 2g). In agreement with the results found in the pine processionary bioassay described above, the results of the cafeteria test showed strong and significant differences (χ^2 = 29.95, df = 3, P < 0.001; Appendix S3: Table S2) on consumed needles (Fig. 2h), where unparasitized (51.6 ± 6.7 mm) and pines with low mistletoe load
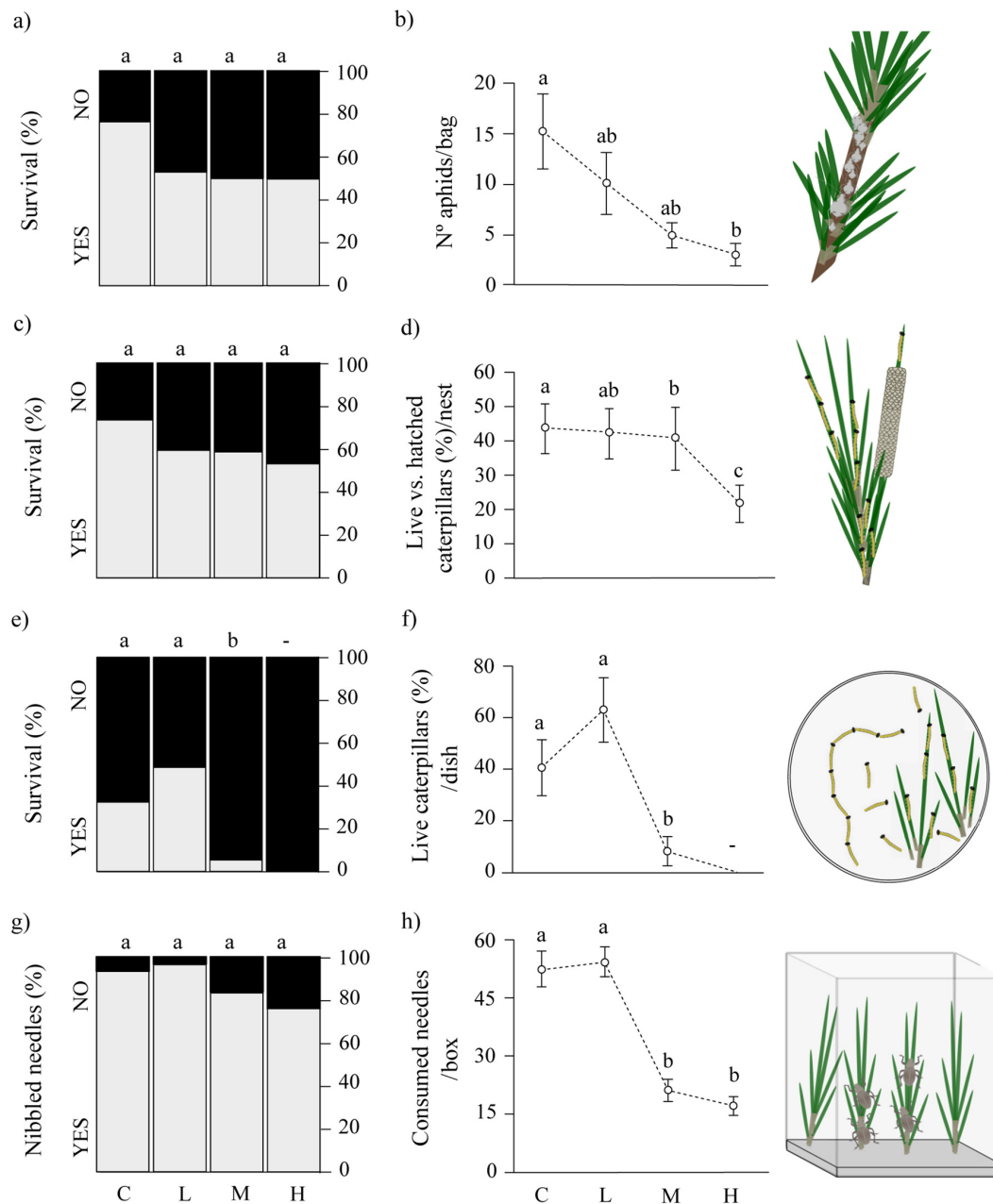
Fig. 2. Pine-feeding herbivores in experimental conditions: survival (left column, gray and black bars, respectively) and abundance (right column, mean ± SE) of the three target herbivore species, according to the mistletoe loads on the pine host (C = control, L = low, M = medium, and H = high). Survival rates show the percentage of (a) bags inhabited by at least two live aphids (n = 157), (c) nests and (e) Petri dish (n = 148) with any live pine processionary caterpillars (n = 127), during field and laboratory bioassays, respectively, and (g) pine needles per box (n = 120) nibbled by pine weevils during cafeteria test. Abundance column shows (b) number of aphids inside the bags, (d) percentage of live vs. hatched pine processionary caterpillars inside the nests, (f) percentage of live out of 20 pine processionary caterpillars per Petri dish, and (h) pine needles consumed (mm) by the pine weevils. Differences among parasitism levels are indicated by different lower-case letters.

(55.3 ± 5.6 mm) were preferred over pines with medium (23.4 ± 3.9 mm) and high parasite loads (17.4 ± 3.1 mm).

Discussion

Here, we demonstrate, by sampling wild populations and by field and laboratory experiments, that mistletoe-induced indirect interactions reduced abundance of several insect herbivores sharing the same host. In addition, the intensity of this detrimental effect has a non-proportional relation to parasite loads, insect herbivores feeding on medium and highly parasitized pines being more affected by decreasing their presence and abundance. Thus, the impact of mistletoe on the herbivores sharing the host plant is more evident when hemiparasite biomass is at a maximum (see Ewald et al. 2011 for a similar result). Therefore, consequences for insect herbivore performance depended basically on the parasite load, because prolonged and heavy parasitism (medium and high parasite loads) affects pines more severely, diminishing their needle N content and increasing defense compounds (Lázaro-González et al. 2019).

We identified the indirect effects of mistletoe on arthropod herbivores as a TMII, via changes in the quality (trait) of the pine as food. The changes caused by mistletoe parasitism in pine (Zweifel et al. 2012, Scalon and Wright 2015) transform pine tissues into a worse food for the insect due to stronger induced defenses (Lázaro-González et al. 2019) and a reduction of mineral nutrients (Mutlu et al. 2016). These negative effects of mistletoe on pines prove especially stressful at a site such as Sierra de Baza, with poor soils (Mellado et al. 2016) and a severe summer drought, where high mistletoe load inevitably leads to the death of the pine (Mellado and Zamora 2016). When the parasitism is not yet massive, although the pine remains alive, it already shows changes that negatively affect the herbivores that feed on the pine. Consequently, we confirm that mistletoe parasitism generates non-trophic links by producing TMIIs and non-proportional effects on pine-feeding herbivores of a shared host.

The effect of mistletoe on pine herbivores across its shared host (Fig. 3) includes non-trophic connections and three different links.

The first interaction (link 1 in Fig. 3), widely reported in the literature, is a direct and top-down effect, or in parallel effects, depending on whether mistletoe is considered a herbivore (Pennings and Callaway 2002) or a plant, respectively, caused by a hemiparasitic plant (Viscum album) on a host plant (Pinus nigra) removing nutrients (Mutlu et al. 2016) and altering their concentrations of chemical defenses (Lázaro-González et al. 2019). These effects suggest that mistletoe reduces pine quality as food, regardless of the tissue considered (phloem or needles). This idea is confirmed by the fact that all herbivores used in our trials, both in field and in laboratory experiments, proved consistently detrimental. This is noteworthy because sometimes a benefit has been suggested for herbivores when the nutrient plant undergoes some type of stress, for example, due to parasitism (Schwartz et al. 2003). In our case, the pine processionary moth and pine weevil appeared to benefit from low levels of mistletoe load (Fig. 2e–h), although without significant differences with the unparasitized pines, and by contrast they are clearly harmed by medium or high parasitism. Also noteworthy is the marked increase in the natural populations of aphids in highly parasitized pines (though without significant differences with others level of mistletoe load, Fig. 1a,b), although this appears rather to be an effect of the reduction in natural enemies. It is known that parasitic plants interfere with the emission of volatile defense compounds produced by the plant against herbivory (Runyon et al. 2008). The contrast of the censuses of natural populations with those of the bioassay, in which natural enemies are totally excluded, points in this direction.

The second link (link 2 in Fig. 3) is the next direct and bottom-up effect above host-feeding herbivores, represented by different guilds (Cinara pini, Thaumetopoea pityocampa, and Brachyderes sp.), where their responses vary according to the mobility of each herbivore species. On the one hand, we found lower survival rates in the aphid and caterpillar populations due to their scant ability to switch from parasitized to unparasitized pines. On the other hand, pine weevils changed their selection, on detecting unpalatable food in parasitized trees and leaving it in order to
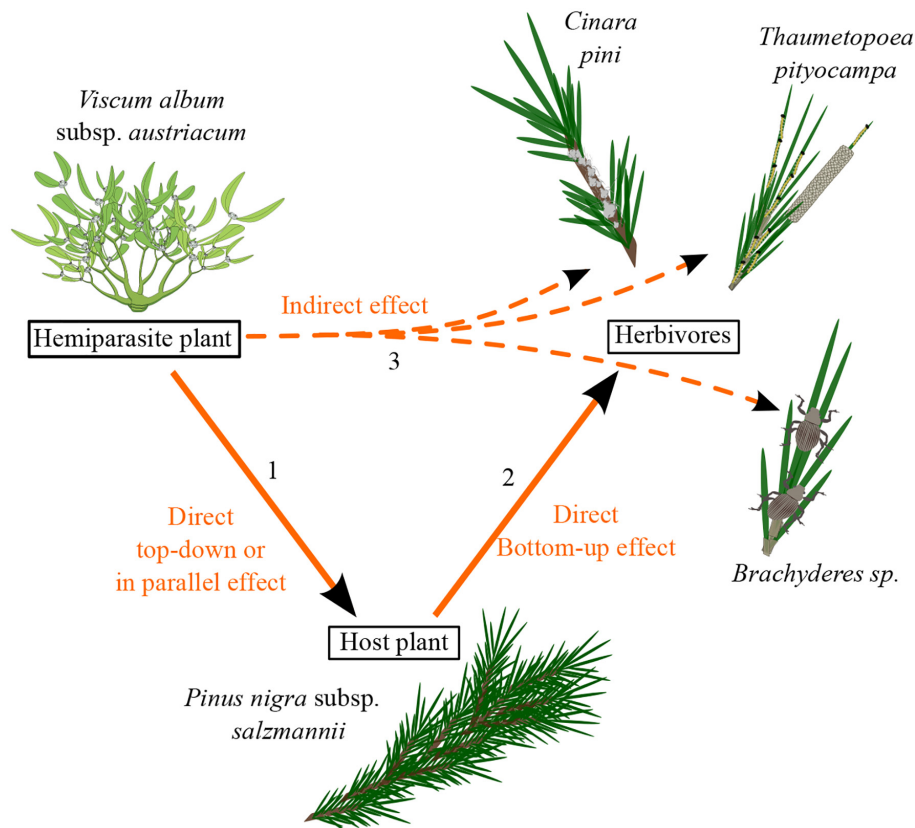
Fig. 3. Scheme of effects of mistletoes on pine-feeding herbivores across its shared host.

find and choose a mistletoe-free tree. Therefore, the last link corresponding to TMIIs (link 3 in Fig. 3) appears when *V. album* competes indirectly with host-feeding herbivores for a shared resource (Puustinen and Mutikainen 2001, Press and Phoenix 2005).

Mistletoes triggered negative TMIIs through pines, but only with medium and high parasite loads, given that in all cases pines with low parasitism acted as unparasitized pines. For pine-feeding folivores, a medium parasite load means a threshold where their survival or abundance will decrease significantly, while for sap-sucking herbivores, this decrease will be gradual as the parasite load increases, being affected significantly only at high parasitism levels. In other words, the relationship between parasite load and their intensity is non-proportional. Despite the general pattern of more detrimental effects for herbivores as mistletoe parasitism intensifies, there are some response differences between folivores and sapsuckers. *Cinara pini* is less sensitive to the parasitism threshold, being affected only by high parasitism levels. By contrast, pine folivores, represented by *T. pityocampa* and *Brachyderes* sp., show lower tolerance to mistletoe parasitism than did sap-sucking herbivores, being affected by the medium parasite load. Thus, in all cases the insect population diminished in parasitized trees, although, in agreement to our predictions, the response differed between FFGs, being stronger on folivorous herbivores than on sap-sucking ones. Previous studies have stated that sharing a host plant with a hemiparasite may be strongly detrimental (Hartley et al. 2007, Bass et al. 2010) and, on the contrary, can generate some benefits for insect herbivores from different FFGs (Ewald et al. 2011, Hartley et al. 2015). In all cases, the effects of these indirect interactions are context-dependent and frequently difficult to explain. Therefore, in our

case, it is essential to determine and consider the parasite load, the FFG, and the mobility of the host-feeding herbivore in order to describe accurately the TMIIs effects on mistletoe–host–herbivores system.

Overall, our results suggest that folivores are more responsive than sapsuckers, or mistletoes are causing more severe changes in pine needles than in phloem. We suggest that needles become a low quality resource, due to changes in chemical defense of pine needles to mistletoe parasitism (Lázaro-González et al. 2019), resembling a folivore attack. Meanwhile, although phloem may contain less nutrients on parasitized pines, it could be lacking defense compounds. For these reasons, more studies delving into the chemical-profile change in different pine tissues are required in order to understand the mechanisms leading to herbivore-feeding responses.

In summary, the present study shows that mistletoe competes against arthropod herbivores and causes a decrease on their populations via a TMII mediated by shared pine resource. In this way, the global impact of consumers on pines is not the addition of negative partial effects of mistletoe and insect herbivores attack, but rather an interaction between biotic stressors. Thus, mistletoe becomes a determinant of the abundance of some species of host-feeding herbivores, relieving the host pines of many of them. This event becomes critical since the mistletoe acts indirectly as a regulator of one of the most severe defoliating plagues in the Mediterranean pine forests, the pine processionary moth. In fact, we show that a pine parasitized by mistletoe is unlikely to be attacked by the processionary. Consequently, indirect mistletoe interaction diminishes host stress and consumption intensity by herbivores, and thus lowers the probability of tree death, thereby helping to lengthen the parasite's own lifespan.

While mistletoe attracts a new community of specialists, different from host pine (Lázaro-González et al. 2017), the pine-feeding herbivore insect populations undergo dramatic and lasting impacts by mistletoe parasitism. These changed assemblages (i.e., detrimental effects on pine herbivores and novel niche for specialized mistletoe fauna) generate a pine canopy, which offers a new combination within the herbivore community to high trophic levels on parasitized pines,

with impact on predators such as insectivorous birds, parasitoids, pathogens, and mutualists associated as ant-tended aphids. All these mechanisms and processes make mistletoe a key species able to reorganize the canopy community of the pine forest, providing a clear demonstration of the importance of indirect interactions as major structuring forces in plant–animal interactions.

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