

## GROWTH OF HARTWEG'S PINE (*PINUS HARTWEGII*) PARASITIZED BY TWO DWARF MISTLETOE SPECIES (*ARCEUTHOBIMUM* SPP.)

MÓNICA E. QUEIJEIRO-BOLAÑOS AND ZENÓN CANO-SANTANA<sup>1</sup>

Departamento de Ecología y Recursos Naturales, Facultad de Ciencias,  
Universidad Nacional Autónoma de México, México D.F., Mexico

<sup>1</sup>Corresponding author: zcs@ciencias.unam.mx

**Abstract:** Coniferous forests occupy a large extent (17 %) of the Mexican territory. Within these forests, pines are a common and sometimes dominant component; however, several abiotic and biotic factors affect pines growth. Among the main biotic factors is the parasitic effect of dwarf mistletoes. In Zoquiapan (Iztaccíhuatl Popocatepetl National Park, Central Mexico) two dwarf mistletoe species coexist parasitizing *Pinus hartwegii*. The aim of this study was to know the effect of *Arceuthobium globosum* and *A. vaginatum*, either individually or as a pair, on *P. hartwegii* growth, allometric relations, and size susceptibility. We recorded diameter at breast height (dbh) and crown spread of *P. hartwegii* for 3 years on individuals infested by either one of the species, both, or none, as well as the infection severity. The relative growth rate (RGR) in diameter was strongly influenced by the pines initial dbh; whereas the infecting species or severity did not show a differential effect. The allometric relation of dbh and height was affected by parasitism, where the trees infected by both species were shorter than the uninfected and infected by *A. vaginatum* at the same dbh. The parasitic effect does not differ among these mistletoe species. However, the host-size structure affects the presence and severity of infection; maintaining even-age stands provides a better scenario for a milder effect of parasitism, which should be considered for managing plans.

**Key words:** allometry, *Arceuthobium globosum*, *Arceuthobium vaginatum*, parasitic plants, pines, relative growth rate.

**Resumen:** Los bosques de coníferas ocupan una gran extensión en el territorio mexicano (17 %). Dentro de estos bosques, los pinos son componente comunes, y en ocasiones dominantes; sin embargo, diversos factores abióticos y bióticos afectan su crecimiento. Entre los principales factores bióticos se encuentra el efecto parasítico del muérdago enano. En Zoquiapan (Parque Nacional Iztaccíhuatl Popocatepetl, Centro de México) dos especies de muérdago enano coexisten y parasitan a *Pinus hartwegii*. El objetivo de este estudio es el de conocer el efecto de *Arceuthobium globosum* y *A. vaginatum*, solos o en conjunto, en el crecimiento de *P. hartwegii*, las relaciones alométricas y la susceptibilidad por tamaños. Registramos el diámetro a la altura del pecho (dap) y la dispersión de la copa de *P. hartwegii* por tres años en individuos infestados por alguna de las dos especies, ambas o ninguna, así como la severidad de la infestación. La tasa relativa de crecimiento (TRC) en diámetro estuvo fuertemente influida por el dap inicial de los pinos, mientras que la especie que infecta y la severidad no mostraron un efecto diferencial. La relación alométrica del dap y la altura fue afectada por el parasitismo, ya que, dentro de una misma categoría de dap, los árboles infectados por las dos especies fueron más bajos que los no infectados y los infectados por *A. vaginatum*. El efecto del parásito no difirió entre especies. Sin embargo, la estructura de tamaños del hospedero afecta la presencia y severidad de la infección, por ello, el mantenimiento de bosques de no coetáneos provee un mejor escenario para atenuar el efecto del parasitismo, observación que debe ser considerada en los planes de manejo.

**Palabras clave:** alometría, *Arceuthobium globosum*, *Arceuthobium vaginatum*, plantas parásitas, pinos, tasa relativa de crecimiento.

Coniferous forests represent 17 % of the Mexican territory. About 90 % of this area comprises *Pinus* or *Pinus-Quercus* species association (Rzedowski, 2006). There are approximately 50 pine species described in Mexico, which represents more than 50 % of all pine species around the

world, many of these with distribution restricted to the country (Nieto de Pascual-Pola, 2009; GD, 2013). Moreover, pines can be distributed over a wide variety of climatic, topographic, and geologic conditions, ranging from cold to warm weathers, and from high mountains to the sea

level (Rzedowski, 2006). Hartweg's pine (*Pinus hartwegii* Lindl.) forests are of great ecological importance because they are the arboreal vegetation most tolerant to low temperatures and high altitudes, reaching the treeline limit at 4,000–4,200 m a.s.l. (Musalem and Solís, 2000; Rzedowski and Rzedowski, 2005; Rzedowski, 2006). Therefore, Hartweg's pines are distributed in almost all high mountain areas and peaks of Mexico, and they tend to form large monospecific stands (Musalem and Solís, 2000).

The main biotic agents that impact *Pinus hartwegii* growth are intraspecific and interspecific competition (Zepeda and Villarreal, 1987; Santillán, 1991; Geils and Hawksworth, 2002; Kunstler *et al.*, 2012), seed and cone predation by rodents (Musalem and Solís, 2002), and forest diseases (Musalem and Solís, 2002; Cibrián *et al.*, 2007). Among the principal forest diseases on *P. hartwegii* are enlisted insects, such as the Scolytid bark beetles, Torymidae wasps and Pyralidae moths, fungi (such as *Ganoderma* spp., *Poliporus* spp. and *Amillaria* spp.), and dwarf mistletoes (Musalem and Solís, 2002; Cibrián *et al.*, 2007).

Dwarf mistletoes (*Arceuthobium* spp., Viscaceae) are hemiparasitic plants and one of the most relevant disease causing agents in pines of the North American temperate forests (Shaw *et al.*, 2008; Mathiasen *et al.*, 2008). These parasitic plants are of ecological importance because they play a key role by providing resources, such as shelter and food, to different animal species enhancing community diversity (Watson and Herring, 2012; Chávez-Salcedo, 2013). Moreover, they are of economic importance because their infection causes a reduction in growth and fitness in the host trees leading to a major forest product loss (Hawksworth and Wiens, 1996; Heide-Jorgensen, 2008). In Mexico, there is an annual loss of  $2.0 \times 10^6$  m<sup>3</sup> of roundwood (Vázquez, 1993), whereas the loss in Western USA and Canada has been estimated as  $11.3 \times 10^6$  m<sup>3</sup> and  $3.8 \times 10^6$  m<sup>3</sup>, respectively (Heide-Jorgensen, 2008).

The stand structure is strongly associated with dwarf mistletoe dynamics, where it has been shown that the crown volume and size of the dominant cohort decreases with infection intensity (Shaw *et al.*, 2005; Agne *et al.*, 2014). These hemiparasitic plants form a complex root-like structure, called haustorium, which is the organ of contact with the hosts' xylem and phloem (Hawksworth *et al.*, 2002). Through this connection, mistletoes take mineral nutrients, water and, although they have a photosynthetic capacity, most of their organic nutrients (Press, 1995). The latter have important effects on host performance, causing decreased growth, branch and stem deformations, reduced water use efficiency, decreased photosynthetic capacity, decreased fecundity and, with severe infection, death of the host (Geils and Hawksworth, 2002; Meinzer *et al.*, 2004; Mathiasen *et al.*, 2008). The impact can go from negligible to severe, depending on the level of the infection and the development status and vigor of the host

(Musselman and Press, 1995). Some studies report that the effect on the hosts' growth is unnoticeable until the infection is severe (Hawksworth and Wiens, 1996; Geils and Hawksworth, 2002; Shaw *et al.*, 2008).

A decrease in the growth of the host has been recorded for different morphometric variables: diameter at breast height (dbh), tree height, volume, and basal area (Hawksworth and Wiens, 1996; Geils and Hawksworth, 2002; Shaw *et al.*, 2008). Andrade and Cibrián (1980) report a reduction of 19 and 29 % in dbh and height, respectively, in *Pinus hartwegii* individuals parasitized by *Arceuthobium* spp. compared with healthy individuals. In other conifers, the reduction in parasitized individuals' dbh due to different dwarf mistletoe species ranges from 2 to 56 %, where growth decrease is non-linearly related to infection class; that is, slight reduction is produced with light infection, but there is a threshold of severe infection after which reduction increases rapidly (Hawksworth and Wiens, 1996; Geils and Hawksworth, 2002). Similar results have been reported for the hosts' volume and basal area (Hawksworth and Wiens, 1996; Madrigal *et al.*, 2007; Shaw *et al.*, 2008).

Hartweg's pines are not only of ecological importance but also of an economic one (Eguiluz, 1978; Hernández, 1985; Musalem and Solís, 2002). Forest diseases, such as dwarf mistletoes, lessen their health for which it is important to investigate their effect in order to include this last aspect for management and reforestation plans. Although the effect of dwarf mistletoes on host performance has been widely studied, the joint effect of different species parasitizing the same host tree has not been investigated. In Zoquiapan (Central Mexico), two species of dwarf mistletoe, *Arceuthobium globosum* Hawksw. & Wiens and *A. vaginatum* J.Presl., coexist parasitizing *Pinus hartwegii* on the same areas and even on the same individual host, showing an aggregated pattern within the host (Queijeiro-Bolaños *et al.*, 2014). Both mistletoe species have a complex interaction within each other, as they compete for host resources; however, under particular circumstances, they also facilitate each other's colonization (Queijeiro-Bolaños *et al.*, unpub. data). The latter makes us think that the effect of these species parasitism may be different when just one or two species are infecting the host. The objectives of this study were: (1) to evaluate the isolated and the combined effect of two dwarf mistletoe species infestation (*Arceuthobium globosum* and *Arceuthobium vaginatum*) on the growth of *Pinus hartwegii*, (2) to assess whether the allometric relationship between height and diameter-at-breast-height of the pines is modified by the infestation, and (3) to recognize what host size is more susceptible to the infection.

## Material and methods

**Study area.** The study was conducted in the Zoquiapan portion of the Iztaccíhuatl Popocatepetl National Park, State of

México. It is located on the East Central part of the Trans-Mexican Volcanic Belt and comprises the main part of the Sierra Nevada (SEMARNAT, 2013). The study site was located at one side of the Southern slope of the Papayo Hill (19° 18' 08.4" N, 98° 42' 10.7" W, 3,420 m a.s.l.). The climate is temperate sub-humid with summer rains, mean annual temperature is 9.8 °C (range: 1.3-18.3 °C), and annual rainfall is 941 mm, with duration of the rainy season being June to September (SMN, 2013). Vegetation is classified as high mountain temperate forest, dominated by large extensions of *Pinus hartwegii* stands (Arriaga *et al.*, 2002); other arboreal species present are *Abies religiosa*, *Alnus jorullensis*, *Cupressus lusitanica*, *Pinus ayacahuite*, *P. leiophylla*, *P. montezumae*, *P. pseudostrobus*, *Quercus crassipes*, *Q. laurina*, and *Q. rugosa*. Tussocky grasses, such as *Muhlenbergia macroura*, *M. quadridentata*, *Calamagrostis tolucensis*, and *Festuca tolucensis*, are the most frequent and conspicuous plant species on the understory (Obieta and Sarukhán, 1981; SEMARNAT, 2013).

*Study species.* Hartwegs' pine or Mexican mountain pine, *Pinus hartwegii*, according to Musalem and Solís (2000) and Rzedowski and Rzedowski (2005), is typical of Mexican mountain ranges on altitudes from 2,600 to 4,200 m, being most frequent on the Sierra Nevada region. It is a tree of 15-30 m in height and up to 75 cm dbh. The bark is thick, rough and scaly, and dark brown to gray in color. Branchlets are stiff, curving upwards with persistent leaf bases. Needles in fascicles are presented in groups of three, although sometimes five, of 6-18 cm length. Cones are grouped in two-six, and are obliquely ovoid, 7-14 cm, and reddish to almost black in color. The seeds are brown, 5-8 mm long, often having black spots, and wings of 12 × 20 mm. Generally, it can be distributed in monotypic stands, but in its lower altitudinal range it can be found coexisting with other trees, such as *Abies religiosa* and *Alnus jorullensis*.

In Zoquiapan, *Pinus hartwegii* is parasitized by *Arceuthobium globosum* subsp. *grandicaule* Hawks. & Wiens and *A. vaginatum* subsp. *vaginatum* (Mexican dwarf mistletoe), which is the zone where the three species converge (Hawsworth and Wiens, 1996; GD, 2013). The two mistletoe species have similar features: dioecious plants, with sexual dimorphism; anemophilous pollination and ballistochoric dispersal; and seven host species in common (*Pinus hartwegii*, *P. durangensis*, *P. lawsonii*, *P. montezumae*, *P. patula*, *P. pseudostrobus*, and *P. rudis*). *Arceuthobium globosum* is 18-70 cm tall with yellow-greenish shoots; it is distributed from Central Mexico to Guatemala. *Arceuthobium vaginatum* is 20-55 cm tall with brown-blackish shoots; it is distributed from Northern to Southern Mexico (Hawsworth and Wiens, 1996; Cibrián *et al.*, 2007).

*Sampling and data analysis.* In a 1.1 ha monotypic stand, we selected pine trees > 2 m and 2.5 cm of diameter at breast

height, because smaller trees are rarely infected by dwarf mistletoe (< 2 %; Hernández-Benítez *et al.*, 2005). The stand has an average of 55.9 % of trees infected by *Arceuthobium globosum* and 22.9 % by *A. vaginatum* (Queijeiro-Bolaños *et al.*, 2014). A total of 161 pines of different size and infection condition (i.e., pines infected by *A. globosum*, *A. vaginatum*, both species, or neither) were selected. From November 2008 to November 2011, we measured diameter at breast height (dbh), tree height, and average crown spread for each individual, every six months. The average crown spread was obtained by measuring the longest extent of the crown and the longest cross-extent, and then averaging the two measures (Blozan, 2004). The height was estimated by calculating the angle ( $\alpha$ ) with a clinometer from the observer to the tip of the tree, and then the distance (d) from the observer to the tree. The height was estimated by using the formula  $h = d \times \tan(\alpha)$ .

We also measured the infection intensity with a modified dwarf mistletoe rating system (MDMR), based on Hawsworth's method (Hawsworth, 1977). In our system, the tree was vertically divided into thirds, and each third was rated into one of the following classes: 0, no infection; 1, sprout or hypertrophy; 2, less than 20 % of branches infected; 3, 21 to 30 %; 4, 31-50 %; 5, 51-70 %; and 6, more than 71 %. The three thirds were summed for each individual, so the scale went from 0 to 18.

To evaluate the isolated and the joint effect of the dwarf mistletoe infection on *Pinus hartwegii* growth rate, we first tested with repeated measures ANOVA (Zar, 2010) whether there was a significant change in dbh, height, and crown spread over the three years. Because only dbh showed a significant change (Appendix 1), we computed the pines' annual relative growth rate (RGR) using dbh. We first analyzed separately, if there were significant differences between the infecting species (i.e., both species, *Arceuthobium globosum*, *A. vaginatum*, or none) on dbh RGR and initial dbh by one way ANOVA and on MDMR by Kruskal-Wallis test. We then performed a linear model including, as main effects, the infection severity (MDMR) and mistletoe species (none, *A. globosum* alone, *A. vaginatum* alone, or both coexisting mistletoes), and pines' initial dbh as a covariate, as it was found to be related to RGR (See Results section). We used the records of MDMR and infecting species at the beginning of the study, because RGR for the three-year sampling must be a response of the previous conditions of parasitism, and three years is a short time to notice a significant change on MDMR or species present (linear analysis of RGR with final MDMR and species, and also with the average MDMR and species can be found in the Appendix 1).

To determine whether the relationship between height and dbh was modified by the infection, we fitted the Chapman-Richards model (Peng *et al.*, 2001) with non-linear least squares method (*nls*) for the following four types of infection: pines parasitized by *Arceuthobium globosum*, *A.*

*vaginatum*, and both, or neither. Chapman-Richards is a non-linear function that describes the changes in the relative dimensions of different parts of the pines, in this case height and dbh. The simplest form of the equation is as follows:

$$y = \alpha(1 - e^{-\beta x})^\gamma \quad (1)$$

where  $y$  refers to the tree height,  $x$  is dbh, and  $\alpha$ ,  $\beta$ , and  $\gamma$  are the parameters of the asymptote, the rate and the form (Richards, 1959; Chapman, 1961). Peng *et al.* (2001) added dbh (1.3 m) to the function, to ensure that the total height equals 1.3 m when dbh is 0:

$$y = 1.3 + \alpha(1 - e^{-\beta x})^\gamma \quad (2)$$

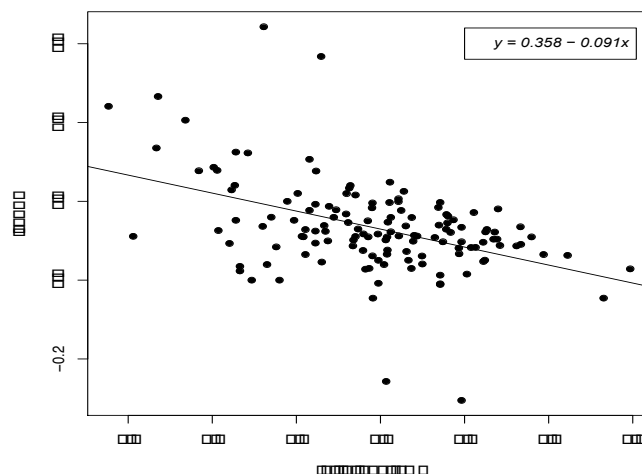
The parameters of the four fitted curves were tested against a null model where the data sets of each condition (none, both species, *Arceuthobium globosum*, or *A. vaginatum*) shared global parameters (i.e., a single curve for all the data), and tested with an extra sum of squares  $F$  test (Motulsky and Christopoulos, 2003) with Prism 6 (Graph-Pad Software, 2014).

In order to know which host size is more susceptible to infection, either by one species or both, we prepared  $7 \times 4$  contingency tables, with seven size categories based on crown spread (height and dbh were non-significant; hence it is not shown in the results) and four infection conditions (i.e., infecting species: no infection, *Arceuthobium globosum*, *A. vaginatum*, or both species). Moreover, we prepared a  $7 \times 4 \times 2$  contingency table with the same seven size classes, four levels of infection [based on MDMR values: none, light (rates 1, 2, and 3), moderate (4, 5, and 6), and severe (7, 8, 9, and 10, because rates 11 to 18 were not found)], and two dwarf mistletoe species. For each contingency table, we performed a generalized linear model (glm) with Poisson error distribution and log link function (Crawley, 2007).

For tridimensional contingency tables, we first fitted the saturated model, where the main effects and the two and three-way interactions were present. Later, we compared it with models lacking the three and two-way interactions with a log-likelihood test (Crawley, 2007). Thereafter, we verified through Pearson residuals whose categories were significant. All analyses were performed using the *stats* package, except for Pearson residual analysis, which was performed using the *vcd* package (Meyer *et al.*, 2006, 2012; Zeileis *et al.*, 2007) both of the R software (R Development Core Team, 2012).

## Results

The relative growth rate (RGR) of *Pinus hartwegii* was strongly correlated with the pines initial dbh, where there was a clear tendency of a decrease in RGR as the trees become larger ( $r^2 = 0.2$ ,  $P < 0.001$ ; Figure 1). There were significant differences according to the parasitizing species (both, *Arceuthobium globosum*, *A. vaginatum*, or none), on



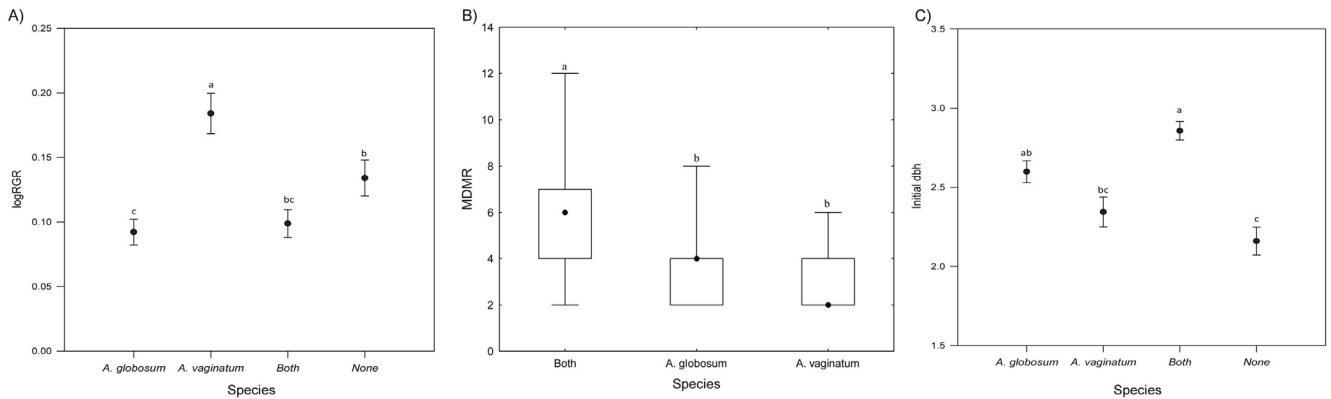
**Figure 1.** Relation of RGR and initial dbh.

dbh RGR ( $F_{(3,123)} = 2.3$ ,  $P < 0.0001$ ; Figure 2A), MDMR ( $\chi^2 = 32.46$ , d.f. = 2,  $P < 0.0001$ ; Figure 2B), and initial dbh ( $F_{(3,126)} = 10.068$ ,  $P < 0.0001$ ; Figure 2C). Trees infected by *A. vaginatum* showed larger RGR, followed by none-infected trees and trees with *A. globosum*, whereas trees with both mistletoes did not differ with those infected with *A. globosum* or uninfected trees (Figure 2A). On the other hand, trees infected by both species had a larger MDMR than each dwarf mistletoe alone (Figure 2B). In addition, the initial dbh of trees with both species was significantly larger than that of trees with *A. vaginatum* alone and the non-infested ones, but did not differ from trees with *A. globosum* alone (Figure 2C). Because we knew that the results of RGR could be misled by the influence of the initial size of the tree, we performed a linear model that included the effect of dbh as a covariate; only the initial dbh turned out to be significant (Table 1). That is, neither the slope nor the intercepts of RGR against initial dbh vary with the infecting species (Table 2, Figure 3). We observed that the RGR of uninfected trees or those infected by both or one species follow the same pattern of decrease with the size described before (Figure 1), suggesting no effect of the parasites.

**Table 1.** Analysis of covariance of the effect of parasitizing species, MDMR, and initial dbh on pines RGR.

	Df	SS	MS	F	P
MDMR	1	0.029	0.029	2.628	0.108
Initial dbh	1	0.349	0.349	31.778	<0.0001
Species	3	0.049	0.016	1.487	0.222
MDMR × dbh	1	0.009	0.009	0.784	0.378
MDMR × Species	2	0.010	0.005	0.453	0.637
Species × dbh	3	0.053	0.018	1.600	0.193
Species × dbh × MDMR	2	0.016	0.008	0.740	0.479
Residuals	121	1.328	0.011		





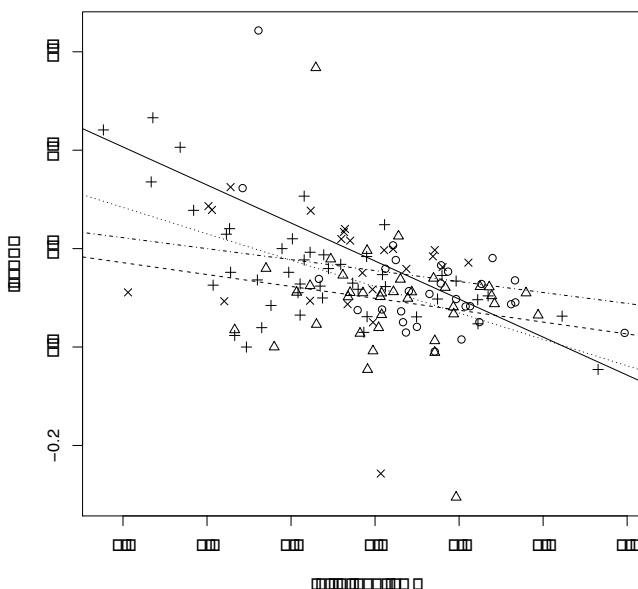
**Figure 2.** Separate effect of parasitizing species, *Arceuthobium globosum*, *A. vaginatum*, both, or none, on the following aspects: A) RGR ( $F_{3,123} = 9.718$ ,  $P < 0.0001$ ); B) mean MDMR ( $F_{2,87} = 26.305$ ,  $P < 0.0001$ ), C) initial size ( $F_{3,126} = 13.649$ ,  $P < 0.0001$ ). Letters above the boxes indicate significant differences according to Tukey's HSD test.

**Table 2.** Summary of the parameters of each level of the factor *Species* on RGR in the model  $RGR = \beta_0 + \beta_1 dbh$ . No significant differences were found.

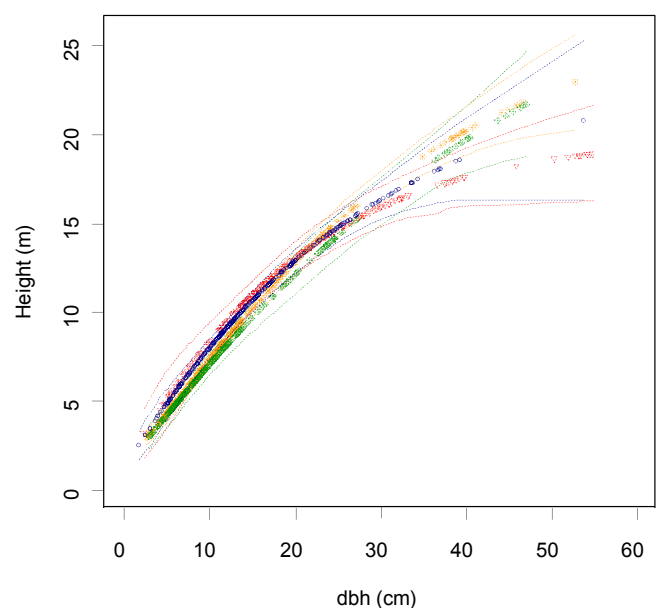
	$\beta_0$	$\beta_1$
<i>Arceuthobium globosum</i>	0.2197	-0.0483
<i>A. vaginatum</i>	0.2667	-0.0445
Both	0.5611	-0.1545
None	0.3910	-0.1073

The allometric relation between tree height and dbh was different for each type of infection (none, both species, *Arceuthobium globosum*, or *A. vaginatum*;  $F_{9,1291} = 2.072$ ,  $P =$

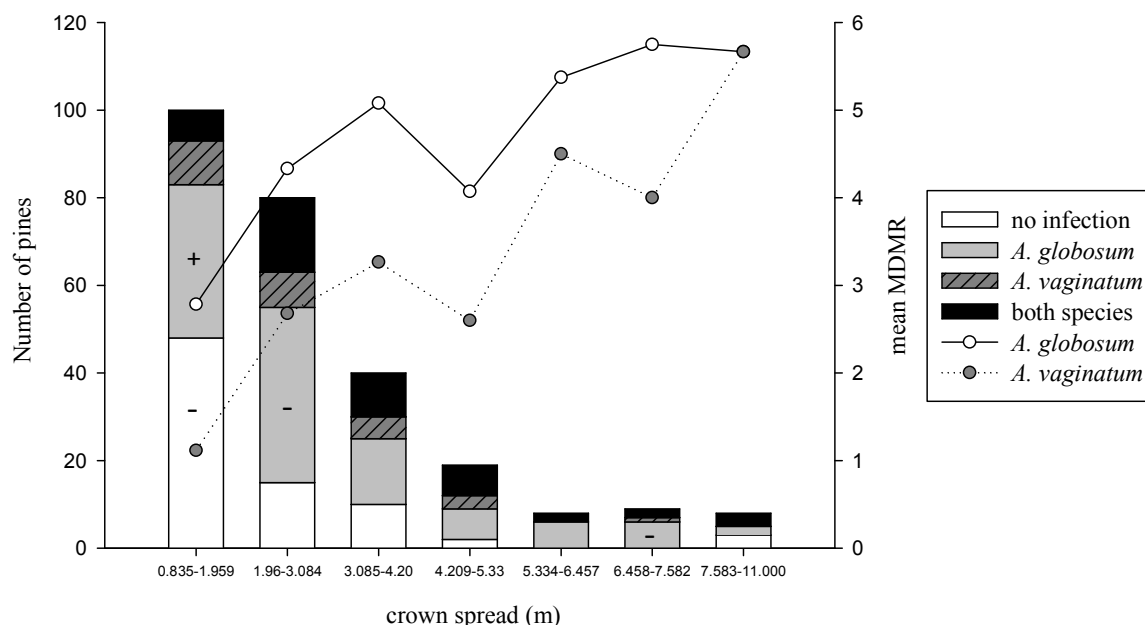
0.029; Figure 4). After fitting the Chapman-Richards model (eq. 2) and plotting the predicted values and their confidence intervals (Figure 4), some patterns could be distinguished. For dbh < 30 cm, there are no clear differences between the infection conditions because the confidence intervals of the four curves overlap; however, on trees > 30 cm dbh, the curves start separating, although their intervals are still overlapped. On trees between 30 and 40 cm dbh, the curve of “both species” begins to look lower than the rest. Between 40 and 60 cm, it is noticeable that the curves of “none” and “*A. vaginatum*” completely overlap, showing no differences in height at those dbh sizes; however, the height of “both species” is considerably lower than the latter two, barely



**Figure 3.** RGR with different initial dbh and different parasitizing species. *Arceuthobium globosum*: Δ symbols and dashed line; *A. vaginatum*: × and dot-dash line; both species: ○ and solid line; none: + and dotted line.



**Figure 4.** Allometric relation between *Pinus hartwegii* dbh and height. Dashed lines represent confidence intervals for each curve; *Arceuthobium globosum*: blue; *A. vaginatum*: orange; both species: red; none: green.



**Figure 5.** Number of pines on different crown classes ( $\chi^2 = 45.421$ , g.l. = 18,  $P < 0.001$ ), parasitized by *Arceuthobium globosum*, *A. vaginatum*, both, or none (left axis), and mean MDMR of *A. globosum* and *A. vaginatum* in this same classes (right axis). (+) symbol indicates a frequency higher than expected, whereas (–) indicates a frequency lower than expected.

overlapping with those two intervals. *Arceuthobium globosum* showed a great variation; although the curve seems to be below the “none” and “*A. vaginatum*” and above both, the confidence interval is too wide to conclude any difference from the other curves.

We tested which crown spread classes were more frequently infected; dbh and height classes were non-significant, hence the results are not reported. In addition, crown spread is a better descriptor for mistletoe presence because it represents the seed “landing” area. There is a significant association between crown spread and infection type ( $\chi^2 = 45.41$ , d.f. = 18,  $P = 0.0003$ ), but only *A. globosum* showed a significant difference between the observed frequencies and that expected by chance in any crown spread size classes (Figure 5), where trees  $< 2$  m are more frequently infected than those expected by this species and trees of 2–3 m and 6.4 and 7.5 had a lower frequency. On the other hand, it is worth to mention that the MDMR increased its magnitude with crown spread size (i.e., the wider the crown, the more severe the infection would be; Figure 5).

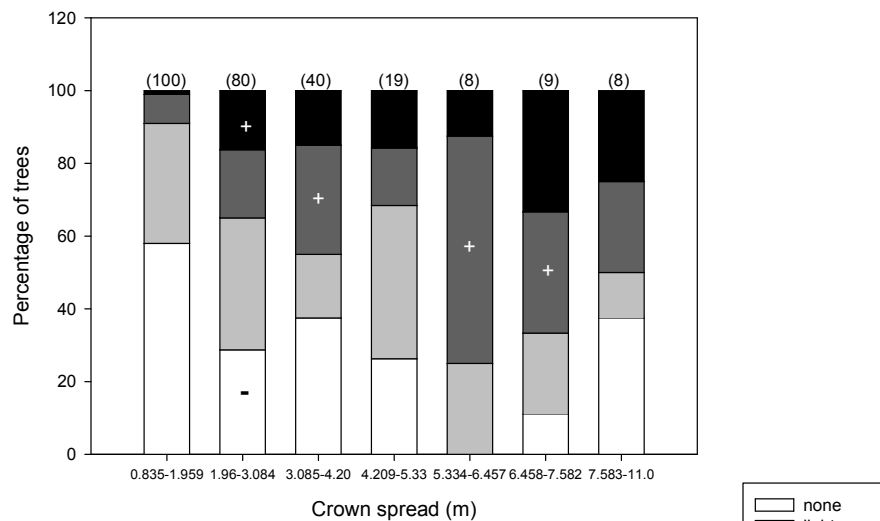
The results of the three dimensional contingency table showed that the three-way interaction between crown spread classes, infection levels (none, light, moderate, and severe), and infecting mistletoe species (*Arceuthobium globosum* and *A. vaginatum*) was non-significant (deviance = 1.19, d.f. = 3, n.s.); nevertheless, the following two-way interactions were significant: infection  $\times$  crown spread class, species  $\times$  infection, and species  $\times$  dbh class (Table A6 on Appendix 1). According to Pearson's standardized residuals, there are some differences between infection level and crown spread ( $\chi^2 = 58.355$ , d.f. = 18,  $P < 0.0001$ ), where cat-

egories moderate or severe of *A. globosum* have, in general, higher observed frequency than expected by chance in trees greater than 1.96 m (Figure 6A), whereas *A. vaginatum* light severity is significantly larger only on trees 1.96–3 m, and underrepresented on trees smaller than 1.96 m (Figure 6B). *Arceuthobium vaginatum* had clearly a lower frequency on *Pinus hartwegii*, and, although non-significantly, has a greater severity on larger trees.

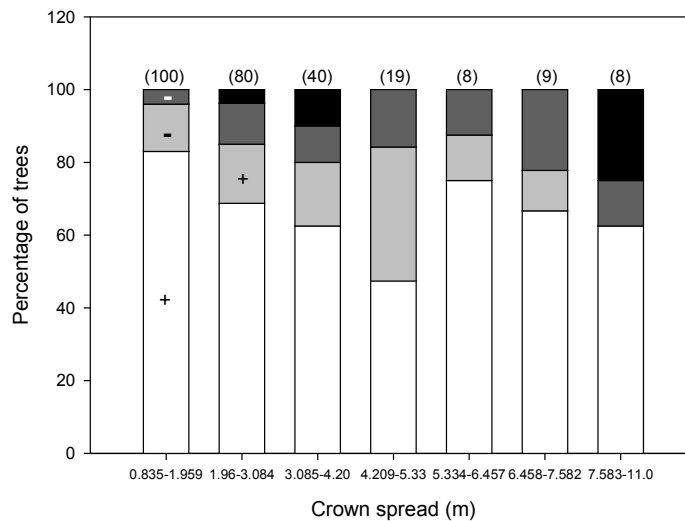
## Discussion

Our results showed that there is no significant effect of the dwarf mistletoe species, either alone or together, on *Pinus hartwegii* dbh RGR; nevertheless, there is evidence that dbh-height allometric relations are different depending on the infecting condition (either *Arceuthobium globosum*, *A. vaginatum*, both, or none). In general, RGR for the four infecting categories follows the same decreasing pattern with dbh. This means that there is no difference between the infected trees (whether these have one or two species) and uninfected ones. The affection of dwarf mistletoes on the hosts' growth has been a common subject of research [see Geils and Hawksworth (2002) for a summary of works]. However, Geils and Hawksworth (2002) acknowledged that only few generalizations can be made for this parasitic genus, where the effect may depend on a mixture of the following factors: i) host-parasite species combination, where some mistletoe species are more harmful than others and some hosts show a higher resistance to infection (Tinnin, 1981; Hawksworth and Wiens, 1996); ii) severity of infection, because the effect can go from negligible with low intensity to

A) *A. globosum*



B) *A. vaginatum*



**Figure 6.** The percentage of *Pinus hartwegii* trees on different crown classes and their infection severity (none, light, moderate, or severe) of the following species: A) *Arceuthobium globosum* and B) *A. vaginatum*. (+) symbol indicates a frequency higher than expected, whereas (–) indicates a frequency lower than expected.

lethal with a large load of parasites (Musselman and Press, 1995; Shaw *et al.*, 2008); iii) host vigor and developmental stage (Tinnin, 1981; Hawksworth and Wiens, 1996); and iv) density-dependent mechanisms and the activity of secondary parasites (Tinnin, 1981).

In the present case, contrary with what has been stated by other authors (Andrade and Cibrián, 1980; Madrigal *et al.*, 2007), it seems that these species, and their combination, are not harmful to pines growth, because there is plenty of evidence that growth is arrested only by age (represented as initial dbh); RGR varies with the ontogenetic condition of the individuals, because there is a decrease in growth rates as the plants increase in biomass (Paine *et al.*, 2012), and this should be considered when modeling plant growth

rates. The influence of the initial dbh on some growth measurement has been treated by other authors; for example, Shaw *et al.* (2008) found that the initial dbh of *Pseudotsuga menziesii* infected with *Arceuthobium tsugense*, explained a considerable amount of the variation in the basal area growth of infected trees.

It is worth mentioning that most studies reporting on the growth effect were conducted on even age (or even sized) stands, which provide a control of some variables, such as initial size, but are hardly extrapolated to natural conditions or uneven age stands. We are presenting evidence that patterns drawn for heterogeneous stands can be fairly different from homogeneous stands; moreover, we can argue that hosts growth on a stand with a heterogeneous size structure

is not severely affected by dwarf mistletoes, indicating the convenience of maintaining forest heterogeneity.

Although no significant effect was shown on RGR, the allometric curves showed some interesting patterns. In general, trees infected with *Arceuthobium globosum* and both species were shorter at dbhs > 30 cm, compared with uninfected trees and those infected with *A. vaginatum*. Although we cannot be certain about the history of the development of these trees and what factors could have affected their growth in the past, we can think of it as a consequence of the presence of mistletoe. Dwarf mistletoes can cause an abnormal biomass accumulation, provoking thicker boles due to hypertrophy or more voluminous crowns due to deformations such as witches' brooms (Hawksworth and Wiens, 1996). Moreover, the infection of dwarf mistletoes can modify stand structure, where the dominant and co-dominant cohort of pines tend to be shorter than uninfected ones (Agne *et al.*, 2014). In this pine species, infection is fairly common over the stem and could cause trees to still gain bole thickness as a response to infection but not height compared with non-infected trees. As expected, the lesser height was notorious for pines infected with both species and *A. globosum*; however, trees infected with *A. vaginatum* showed the same pattern than uninfected trees, demonstrating that the effect of this species could be null when it refers to allometric relationships. It is noteworthy that no differences can be depicted on trees < 30 cm dbh, and this is probably because younger individuals have a greater increment in biomass (Paine *et al.*, 2012), and also because the infection is less severe on these individuals (Figure 5). A non-linear effect has been shown before, where the effect of dwarf mistletoes is not noticeable until the infection is severe (Geils and Hawksworth, 2002; Shaw *et al.*, 2008), and we had a bias toward smaller trees with light or moderate infection, but that is a reflection of the natural stand structure.

With respect to crown spread size susceptibility, some patterns agree with what was expected: most of the uninfected trees were the smallest ones and MDMR increased with tree size (Shaw *et al.*, 2005). Young trees are rarely parasitized because they cannot provide enough resources to the parasite and the consequences for them are mostly lethal (Press, 1995); however, a larger tree represents a better resource because it has a larger surface for seed reception (Arriaga *et al.*, 1988; Hernández-Benítez *et al.*, 2005) and can bear a larger load of parasites declining in health but not in mortality (Musselman and Press, 1995; Hawksworth and Wiens, 1996). From the results, it can be said that trees with a crown spread smaller than 1.96 m were more frequently infected by *Arceuthobium globosum* (Figure 5), but this species is more severely on larger trees (Figure 6); whereas, the categories *A. vaginatum* or both species do not seem to be more or less frequent on any size category (Figure 5), but *A. vaginatum* showed lighter infections on smaller trees and greater infections on larger trees (although this last was not

significant, Figure 6). However, infection of trees with *A. vaginatum* was a lot less frequent than that with *A. globosum*, suggesting that *A. vaginatum* might be less harmful. Forest canopy, here represented as crown spread, represents an important resource for mistletoes because it is not only the place for establishment but it also has the suitable conditions for these plants to perform adequately (Reid *et al.*, 1995; Shaw, 2004). *Arceuthobium vaginatum* is a smaller plant with apparently lesser photosynthetic requirements than *A. globosum* (Calvin *et al.*, 1984; Hawksworth and Wiens, 1996); thus, *A. globosum* could be gaining more benefit from larger trees, hence being more severe within these sizes.

The results showed that these parasites are not significantly harmful for host growth, stating the importance of maintaining forest heterogeneity. This scenario where two dwarf mistletoes are coexisting is rare (Hawksworth and Wiens, 1996; Quejeiro-Bolaños *et al.*, 2014); thus, it raises the question about how resources are used and shared among the two species. In addition, although these parasites may not be arresting pine growth in a noticeable way, we cannot state that these have no effect on forest industry activities, such as poor wood quality and lower roundwood production (Andrade and Cibrián, 1980; Madrigal *et al.*, 2007; Logan *et al.*, 2013).

## Conclusions

Our results suggest that *Arceuthobium globosum* and *A. vaginatum* do not have a joint effect on *Pinus hartwegii* dbh RGR. These lead us to think that the infection by these two parasites on this area is not detrimental for the host populations. Uneven-aged forests represent a more complex scenario where the severity of infection is commonly greater because there are more spaces to colonize (Shaw *et al.*, 2008). Nevertheless, the latter has been reported for single-species stands with the infection of only one mistletoe species; there are two species present in this area using the same host as a resource, and our results suggest that it may be helpful to maintain the complexity of an uneven-age forest to avoid the dominance of only one mistletoe species, which could be more harmful for the host.

## Acknowledgments

UNAM-DGAPA-PAPIIT IN220912 grant to ZC-S funded the study. CONACYT funded MQ-B (grant 220652) for postgraduate studies at the Posgrado en Ciencias Biológicas from the UNAM.

We thank all the people who helped us during field work, Iván Castellanos-Vargas for technical support; Carlos Martorell, Graciela García-Guzmán, and two anonymous reviewers for their helpful comments; and Izta-Popo Zoquiapan National Park office for facilities.



## Literature cited

- Agne M.C., Shaw D.C., Woolley T.J. and Queijero-Bolaños M.E. 2014. Effects of dwarf mistletoe on stand structure of lodgepole pine forest 21-28 years post-mountain pine beetle epidemic in central Oregon. *PLoS ONE* **9**:e107532.
- Andrade V. and Cibrián D. 1980. Evaluación de poblaciones de muérdago enano (*Arceuthobium globosum* Hawks. y Wiens y *A. vaginatum* Willd.) en bosques de *Pinus hartwegii* Lindl en Zoquiapan, Edo. de México. In: Primer Simposio Nacional sobre Parasitología Forestal, Uruapan, Michoacán, México, February 18-19. pp. 238-253.
- Arriaga L., Franco M. and Sarukhán J. 1988. Identification of natural groups of trees in uneven-aged forests using multivariate methods. *Journal of Ecology* **76**:1092-1100.
- Arriaga L., Espinoza J.M., Aguilar C., Martínez E., Gómez L. and Loa E. Coords. 2000. *Regiones Terrestres Prioritarias de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico City D.F.
- Blozan W. 2004. Tree Measuring Guidelines of the Eastern Native Tree Society. <[http://www.nativetreesociety.org/measure/Tree\\_Measuring\\_Guidelines-revised1.pdf](http://www.nativetreesociety.org/measure/Tree_Measuring_Guidelines-revised1.pdf)> (accessed November 15, 2014)
- Calvin C.L., Hawksworth F.G. and Knutson D.L. 1984. Phloem in *Arceuthobium globosum* (Viscaceae). *Botanical Gazette* **145**:461-464.
- Chávez-Salcedo L.F. 2013. Estructura de la comunidad de artrópodos asociados a dos especies de muérdago enano (*Arceuthobium* spp.) parásitos de *Pinus hartwegii* (Lindl.) en el Parque Nacional "Zoquiapan y Anexas." B.Sc. Dissertation, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City D.F. 94 pp.
- Chapman D.G. 1961. Statistical problems in dynamics of exploited fisheries populations. In Neyman J. Ed. *Procedures of the 4th Berkeley Symposium on Mathematical Statistics and Probability*, Berkeley.
- Cibrián D., Vázquez I. and Cibrián J. 2007. Muérdagos enanos del género *Arceuthobium*. In: Cibrián-Tovar D., Alvarado D. y García E. Eds. *Enfermedades Forestales de México*, pp. 357-395. Universidad Autónoma de Chapingo, Chapingo.
- Crawley M.J. 2007. *The R book*. John Wiley & Sons, Chichester.
- Eguiluz-Piedra T. 1978. Ensayo de integración sobre los conocimientos del género *Pinus* en México. B.Sc. Dissertation. Escuela Nacional de Agricultura, Chapingo, Mexico State, Mexico, 517 pp.
- GD, The Gymnosperm Database. 2013. Conifers of Mexico. <[conifers.org](http://conifers.org)> (accessed June 1, 2013).
- Geils B.W. and Hawksworth F.G. 2002. Damage, effects, and importance of dwarf mistletoes. In: Geils B.W. Cibrián J. and Moody B. Eds. *Mistletoes of North American Conifers*, General Technical Report RMRS-GTR-98, pp. 57-66, United States Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- GraphPad Software, Inc. 2014. GraphPad Prism version 6.00 for Windows. La Jolla, California USA, <<http://www.graphpad.com/scientific-software/prism/>>
- Hawksworth F.G. 1977. The 6-class dwarf mistletoe rating system, Volume 48 of General technical report. Forest Service, United States Department of Agriculture, Fort Collins.
- Hawksworth F.G. and Wiens D. 1996. *Dwarf Mistletoes: Biology, Pathology, and Systematics*. Forest Service, United States Department of Agriculture, Washington D.C.
- Hawksworth F.G., Wiens D. and Geils B.W. 2002. *Arceuthobium* in North America. In: Geils B.W. Cibrián-Tovar J. and Moody B. Coordinadores. *Mistletoes of North American Conifers, General Technical Report*. pp. 29-56. United States Department of Agriculture, Fort Collins.
- Heide-Jørgensen H. 2008. *Parasitic Flowering Plants*. Brill Academic Publishers, Leiden.
- Hernández H. 1985. Variación natural de *Pinus hartwegii* Lindl.: dimensiones transversales de las traqueidas en un transecto altitudinal de Zoquiapan. B.Sc. Dissertation. División de Ciencias Forestales, Universidad Autónoma de Chapingo, Chapingo, Mexico State, Mexico. 59 pp.
- Hernández-Benítez R., Cano-Santana Z. and Castellanos-Vargas I. 2005. Incidencia de infestación de *Arceuthobium globosum grandicaule* (Hawks. y Wiens) en *Pinus hartwegii* (Lindl.). *Ciencia Forestal en México* **30**:79-86.
- Kunstler G., Albert C.H., Courbaud B., Laverne S., Thuiller W., Vieilledent G., Zimmermann N.E. and Coomes D.A. 2011. Effects of competition on tree radial-growth vary in importance but not in intensity along climatic gradients. *Journal of Ecology* **99**:300-312.
- Logan B.A., Reblin J.S., Zonana D.M., Dunlavy R.F., Hricko C.R., Hall A.W., Schmiede S.C., Butschek R.A., Duran K.L., Emery R.J.N., Kurepin L.V., Lewis J.D., Pharis R.P., Phillips N.G. and Tissue D.T. 2013. Impact of eastern dwarf mistletoe (*Arceuthobium pusillum*) on host white spruce (*Picea glauca*) development, growth and performance across multiple scales. *Physiologia Plantarum* **147**:502-513.
- Madrigal S., Vázquez I. and Velasco E. 2007. Obtención de parámetros dasométricos para evaluar efecto causado por *Arceuthobium vaginatum* en *Pinus hartwegii* del Nevado de Colima. In: Memorias del VIII Congreso Mexicano de Recursos Forestales, Morelia, Michoacán, Mexico, October 28-31, 2007. pp 1-7.
- Mathiasen R.L., Shaw D., Nickrent D. and Watson D. 2008. Mistletoes. Pathology, systematics, ecology, and management. *Plant Disease* **92**:988-1006.
- Meinzer F.C., Woodruff D.R. and Shaw D. 2004. Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. *Plant, Cell and Environment* **27**:937-946.
- Meyer D., Zeileis A. and Hornik K. 2006. The strucplot framework: visualizing multi-way contingency tables with vcd. *Journal of Statistical Software* **17**:1-48.
- Meyer D., Zeileis A. and Hornik K. 2012. vcd: Visualizing Categorical Data. R package version 1.2-13.
- Motulsky H. and Christopoulos A. 2003. *Fitting Models to Biological Data Using Linear and Nonlinear Regression. A Practical Guide to Curve Fitting*. GraphPad Software Inc, San Diego.
- Musálem-Santiago M.A. and Solís-Pérez M.A. 2000. *Monografía de Pinus hartwegii*. Libro Técnico No. 3, División Forestal, Secretaría de Agricultura, Ganadería y Desarrollo Rural / Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias / Centro de Investigación Regional del Centro Chapingo.
- Musselman L. and Press M. 1995. Introduction to parasitic plants. In: Press M. and Graves J. Eds. *Parasitic Plants*, pp. 1-13, Chapman & Hall, London.
- Nieto de Pascual-Pola M.C.C. 2009. Coníferas. In: Ceballos G., List R, Garduño G., López-Cano R. Muñozcano-Quintanar M.J., Collado E. and San Román J.E. Compiladores. *La Diver-*

- sidad Biológica del Estado de México, pp. 177-185, Gobierno del Estado de México / Comisión Nacional para el Conocimiento y Uso de la Biodiversidad / Universidad Nacional Autónoma de México / Instituto de Ecología, Toluca.
- Obieta C. and Sarukhán J. 1981. Estructura y composición de la vegetación herbácea de un bosque uniespecífico de *Pinus hartwegii*. I. Estructura y composición florística. *Boletín de la Sociedad Botánica de México* **41**:75-125.
- Paine C.E.T., Marthews T.R., Vogt D.R., Purves D., Rees M., Hector A. and Turnbull L.A. 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution* **3**:245-256.
- Peng C., Zhang L. and Liu J. 2001. Developing and validating nonlinear height/diameter models for major tree species of Ontario's boreal forests. *Northern Journal of Applied Forestry* **18**:87-94.
- Press M.C. 1995. Carbon and nitrogen relations. In: Press M.C. and Graves J.D. Eds. *Parasitic Plants*, pp. 103-124, Chapman & Hall, London.
- Quejeiro-Bolaños M., Cano-Santana Z. and García-Guzmán G. 2014. Incidence, severity and aggregation patterns of two sympatric dwarf mistletoe species (*Arceuthobium* spp.) in Central Mexico. *European Journal of Forest Research* **133**:297-306.
- R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<http://www.r-project.org>>
- Reid N., Stafford Smith M. and Yan Z. 1995. Ecology and population biology of mistletoes. In: Lowman M.D. and Nadkarni N.M. Eds. *Forest Canopies*, pp. 285-310, Academic Press, San Diego.
- Richards F.J. 1959. A flexible growth function for empirical use. *Journal of Experimental Botany* **10**:290-301.
- Rzedowski J. 2006. *Vegetación de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico City D.F.
- Rzedowski G.C. de, Rzedowski J. and contributors. 2005. *Flora Fanerogámica del Valle de México*. Instituto de Ecología, A.C. and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro.
- Santillán-Pérez J. 1991. Silvicultura de las coníferas de la Región Central. M.Sc. Dissertation. División de Ciencias Forestales, Universidad Autónoma de Chapingo, Chapingo.
- SEMARNAT [Secretaría del Medio Ambiente y Recursos Naturales]. 2013. Acuerdo por el cual se da a conocer el resumen del Programa de Manejo del Parque Nacional Iztaccíhuatl-Popocatepetl. *Diario Oficial de la Federación*. 2 de marzo del 2013.
- Shaw D.C. 2004. Vertical Organization of Canopy Biota. In: Lowman M.D. and Rinker H.B. Eds. *Forest Canopies*, pp. 73-101, Elsevier Academic Press, Burlington.
- Shaw D.C., Chen J., Freeman E.A. and Braun D.M. 2005. Spatial and population characteristics of dwarf mistletoe infected trees in an old-growth Douglas-fir western hemlock forest. *Canadian Journal of Forest Research* **35**:990-1001.
- Shaw D.C., Huso M. and Bruner H. 2008. Basal area growth impacts of dwarf mistletoe on western hemlock in an old-growth forest. *Canadian Journal of Forest Research* **38**:576-583.
- SMN, Servicio Meteorológico Nacional. 2013. Normales climatológicas de México. <<http://smn.cna.gob.mx/>> (accessed June 1, 2013).
- Tinnin R.O. 1981. Interrelationship between *Arceuthobium* and its host. *American Midland Naturalist* **106**:126-132.
- Vázquez C.I. 1993. *Contribución al conocimiento del muérdago verdadero (Psittacanthus spp.) en México*. Boletín Técnico No. 11. Centro de Investigaciones del Pacífico Centro, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Secretaría de Agricultura y Recursos Hidráulicos, Uruapan.
- Watson D.M. and Herring M. 2012. Mistletoe as a keystone resource: an experimental test. *Proceedings of the Royal Society B*. **279**:3853-3860.
- Zar J.H. 2010. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River.
- Zeileis A., Meyer D. and Hornik K. 2007. Residual-based shadings for visualizing (conditional) independence. *Journal of Computational and Graphical Statistics* **16**:507-525.
- Zepeda E.M. and Villarreal M.E. 1987. *Guía de Densidad para Pinus hartwegii Lindl., de Zoquiapan, México*. Universidad Autónoma de Chapingo, Chapingo.

Received: September 28th, 2014

Accepted: December 23th, 2014

**Appendix.** To test if there were any significant growth of pines dbh, height and crown spread we tested the change in time of aforementioned variables with repeated measures ANOVA. Only dbh changed significantly with time; because of this result, we computed RGR only for dbh. Boldface *p*-values are <0.05.

Table A1. Repeated measures ANOVA of dbh (cm) in time.

	SS	df	MS	<i>F</i>	<i>p</i>
Time (semesters)	423.3	6	70.6	79.734	<b>&lt;0.001</b>
Error	833.5	942	0.9		

Table A2. Repeated measures ANOVA of height (cm) in time.

	SS	df	MS	<i>F</i>	<i>p</i>
Time (semesters)	51028	6	8504.6	1.924	0.074
Error	4242752	960	4419.5		

Table A3. Repeated measures ANOVA of crown spread (cm) in time.

	SS	df	MS	<i>F</i>	<i>P</i>
Time (semesters)	1569.2	6	261.536	1.063	0.383
Error	171190.1	696	245.963		

We considered that the three-year RGR is reflecting the results from the initial infection. Nevertheless, we also tested the effect of final infection (final records of MDMR and infecting species), as well as mean infection (mean MDMR and infecting species). The three analyses (initial MDRM, final MDMR and mean MDRM) showed that initial dbh is the main factor affecting RGR. Boldface *p*-values are <0.05.

Table A4. Summary of the effects of initial dbh and the different levels of final infecting species.

	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	0.399	0.046	8.574	<b>&lt;0.001</b>
Initial dbh	-0.097	0.016	-6.106	<b>&lt;0.001</b>
<i>A. globosum</i>	-0.048	0.023	-2.046	<b>0.043</b>
None	-0.042	0.027	-1.559	0.121
<i>A. vaginatum</i>	0.017	0.030	0.573	0.568

Table A5. Summary of the effects of initial dbh and the different levels of mean infecting species.

	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	0.205	0.025	8.189	<b>&lt;0.001</b>
Initial dbh	-0.005	0.001	-4.363	<b>&lt;0.001</b>
<i>A. globosum</i>	-0.008	0.022	-0.360	0.720
None	-0.009	0.028	-0.325	0.746
<i>A. vaginatum</i>	0.060	0.035	1.681	0.095

To test if two and three-way interaction on three dimensional contingency table were relevant to the model, we performed an ANOVA tests to compare the saturated model (with all possible interactions) against a model without the three-way interaction. Since these models didn't showed a significant difference we kept with the simpler one, which we compared with different models without two-way interactions. Boldface *p*-values are <0.05.

**Appendix.** Continuation.*Table A6. Models comparison for three dimensional contingency tables for crown spread.*

Model 1	Model 2	Deviance	$p(> \chi^2 )$
Saturated (three and two-way interactions included)	Without three way-interaction	-0.948	0.814
Withouth three-way interaction	Without infection×crown class	39.502	<b>&lt;0.001</b>
	Without species×infection	58.540	<b>&lt;0.001</b>
	Without species×crown class	2.890	0.089