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The European Mistletoe Effects on Leaves and Nutritional Elements of Two Host Species in Hyrcanian Forests

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Abstract. For investigation of the mistletoe (*Viscom album* L.) effects on leaf area and weight of the host trees, a number of severely infected individuals and completely healthy trees (control trees) were studied. The area and weight of the eleven largest leaves of branches were collected and measured accurately. Moreover, for comparison of nutritional status, several healthy and infected Hornbeam *Carpinus betulus* and Ironwood *Parrotia persica* species individuals, with approximately similar conditions of DBH, height and other outward features were selected. Almost 100 leaves from parasitized and unparasitized branches of two host species were sampled in order to compare with control trees (uninfected trees near each host). Results showed, the area of leaves and weight of parasitized branches were less than unparasitized branches and control trees in 95% confidence level. Furthermore the amount of mineral nutrients of K, Mn and Zn showed the higher level in parasitized branches compared to control trees; in contrast, the quantity of nitrogen just in Iron wood host species was lower. This paper suggests, the *V. album* L. may have undesirable and displeasing effects on leaf structure, physiological and nutritional status of host trees in high intensity of infections.
Key words: Mistletoe; ironwood species; host trees; nutritional status; leaf area

Efeitos do Visco Europeu nas Folhas e Elementos Nutritivos de Duas Espécies Hospedeiras das Florestas da Hircânia

Sumário. Para investigar os efeitos do visco na área foliar e peso das árvores hospedeiras, estudaram-se indivíduos severamente afectados e totalmente sãos (testemunha). Colheram-se as onze maiores folhas dos ramos e registou-se cuidadosamente a área foliar e o peso. Além disso, para comparação do teor de nutrientes, seleccionaram-se algumas espécies afectadas e algumas sãs, de *Carpinus betulus* e *Parrotia persica*, com semelhante DAP, altura e outras características externas. Amostraram-se cerca de 100 folhas de ramos de árvores parasitadas e não parasitadas, para comparação com as árvores testemunha (árvores sãs próximo de cada hospedeira). Os resultados demonstraram que a área e o peso das folhas de ramos atacados era inferior ao verificado para os de ramos não parasitados e árvores testemunha, para um nível de

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confiança de 95%. Também o nível de nutrientes minerais K, Mn e Zn foi mais elevado em ramos atacados em comparação com as árvores testemunha; pelo contrário, o teor de nitrogénio em *Parrotia persica* foi inferior. Este trabalho sugere que *V. album* L. pode provocar efeitos indesejáveis na estrutura da folha, na fisiologia e no teor de nutrientes das hospedeiras com elevada intensidade de infecção.
Palavras-chave: Visco; hospedeiras; espécies de pau-ferro; teor de nutrientes; área foliar

Effet du Gui d'Européen sur les Feuilles et les Éléments Alimentaires de Deux Espèces Hôtes Dans les Forêts Hyrcaniennes

Résumé. Pour la recherche des effets du gui sur la surface foliaire et le poids des arbres hôtes, un certain nombre d'individus sévèrement infectés et d'autres complètement sains (arbres témoins) ont été étudiés. La surface et le poids des onze plus grandes feuilles des branches ont été recueillis et mesurés avec précision. Par ailleurs, pour la comparaison du statut alimentaire, plusieurs individus d'espèces saines et infectées de Hornbeam et d'Ironwood, avec des DBH approximativement semblables, la taille et d'autres caractéristiques extérieures ont été choisis. Presque 100 feuilles des branches parasitées et non parasitées de deux espèces hôtes ont été prélevées afin de les comparer avec les arbres témoins (arbres non infectés près de chaque hôte). Les résultats ont montré que la surface des feuilles et le poids des branches parasitées étaient moindre que ceux des branches non parasitées ainsi que des arbres témoins, pour un taux de confiance de 95%. En outre la quantité d'aliments minéraux en K, Mn et Zn a montré de plus hauts niveaux dans les branches parasitées comparées aux arbres témoins; mais en revanche, la quantité d'azote, pour *Parrotia persica* était inférieure. Ce document suggère que, *V. album* L. peut exercer des effets indésirables et contrariants sur la structure de la feuille, la physiologie et l'alimentation des arbres de l'hôte dans une forte intensité d'infection.
Mots clés: Gui; espèces d'Ironwood; arbres hôtes; statut alimentaire; région de feuille

Introduction

Mistletoes are the most specious parasitic plants, with some 1400 species from five families within the Santalales order. They are predominant group of angiosperm shoot parasites, are fascinating and diverse group of plants found in wide range of ecosystems including boreal forests, tropical rain forests and arid woodlands (NORTON and CARPENTER, 1998; WASTON, 2001; SHOW *et al*., 2004).

Viscum album L. can manufacture some of its food with the help of nutrients and water absorbed from the host plants though haustoria. The berries of the female plant are small, sticky and whitish and are very attractive to birds such as cedar waxwings, robins and others. The birds feed on and digest the pulp of the berries, excreting the living seeds that stick tightly to any branch on which they land (BARBERAKI and KINTZIOS, 2002; BRIGGS, 2003; WATSON, 2001; ZUBER, 2004).

It has no root system, but obtains water and minerals from the host plant and its green leaves are capable to manufacture food as other green plants (BRIGGS, 2003; ZUBER, 2004). In this work, the amount of some primary mineral nutrients as N, P, K, Ca, Mg, Zn, Mn and Na in the leaves of parasitized and unparasitized branches with *Viscum album* compared with complete healthy trees near them (control trees). We conducted this study to know whether *V. album* may cause reduction of these elements in its two prevalent host species (i.e. *Carpinus betulus* and *Parrotia persica*) in Hyrcanian Forests or not; and either if

mistletoe have the negative effect on leaf area and weight of *Parrotia persica* individuals?

Materials and methods

Study area: Hyrcanian vegetation zone is a green belt stretching over the northern slopes of Alborz mountain ranges and covers the southern coasts of the Caspian Sea. Alborz mountain interception between the Caspian Sea and Iran plateau has resulted in a climate with distinct vegetation cover. Hyrcanian forests stretch out from sea level up to an altitude of 2800 m and encompass different forest type thanks to 80 woody species i.e. trees and shrubs (SAGHEB-TALEBI *et al*., 2005).

In order to perform this study, two indicator community of trees were selected in Hyrcanian forests, because of being the most dominant hosts of *Viscum album* L.. The first, *Parrotio-Carpinetum* community in Nour Forest Park and second, *Querco-Carpinetum* in Educational-Research Forest of Kheiroud kenar-Noshahr.

Investigating the *V. album* effects on leaf area, weight and mineral nutrients content and comparison of parasitized and unparasitized branches of the two host species trees, with leaves of control trees (a very similar tree but not infected, near each of same host species), leaf samples collected from Ironwood trees (*Parrotia persica*) of Nour Forest Park located in 5 Km east of Nour city and Hornbeam (*Carpinus betulus*) samples from 7 Km east of Noshahr city in Kheiroud kenar Forest. Characteristics of these two region prepared in table 1.

Plant material: In order to comparison of mistletoe effects on leaf area and weigh of host trees, five severely infested Iron Wood individuals with five completely health trees (a control tree near each infected one) were selected in Nour Forest Park in August 2004. The sample trees had approximately the same condition of DBH, height, crown figure and health. The parasitized, unparasitized and also the branches of control trees, which selected for sampling, have also similar diameter, length and sun light direction. The eleven largest leaves of branches were collected and area and weight measured accurately. Furthermore, five trees of each host species found in such a manner that have a perfect healthy tree near them. These individuals were without any abnormal symptom such as mechanical lesion or caused by a pest or disease, influence of drought stress and either changing in leaves color. To compare the amount of mineral nutrition, leaf samples picked from unparasitized, parasitized and either healthy trees branches, located in external middle part of the crown with same position of sun light direction.

About 100 perfect leaves (including petiole) picked and placed into tightly sealed nylon bags and immediately transferred to the laboratory with minimum delay to forestall a further change in quality of samples. Then plant materials were oven dried at 65 ˚C for 48 h, ground to a fine powder using a Willey mill and then digested (Wet digestion technique) with H_2SO_4, Salicylic acid, H_2O_2 and Selenium powder. The digest was filtered with Whatman paper no. 42.

Table 1 - Characteristics of the study areas

Region Characteristics	Nour Forest Park	Kheiroud Kenar--Noshahr
Longitude and latitude	36°34'N, 51° 41'E	36°34'N, 51° 50'E
Altitude from sea level	-10 m	640 m
Mean annual rainfall	1040 mm	1345 mm
The mean maximum monthly rainfall	212 mm in Dec.	270.7 mm in Oct.
The mean minimum monthly rainfall	19 mm in Jun.	49 mm in Jul.
Mean annual air temperature	17°C	15.9°C
The mean maximum monthly air temperature	26°C in Jun.	25.2°C in Jul.
The mean minimum monthly air temperature	7.5°C in Jan.	6.7°C in Feb.

Concentrations of the elements were determined using these techniques: total nitrogen with Titration procedure after distillation using KJELTEC Auto Analyzer model Tecator 1030, the potassium and sodium content by flame photometry with Flame Emission Spectrometer model JENWAY Clinical PFP7, phosphorous by Vanodo–Molybdate method using Spectrophotometer model JENWAY 6505 and Ca, Mg, Zn and Mn with Atomic Absorption Spectrophotometer model Shimatsu 6550. Finally all data were analyzed by one-way analysis of variance (ANOVA) and Tukey HSD and Dunnett T3 tests using SPSS 12.0 for windows software.

Results

Mean value comparison of leaf area and weight, showed significant differentce in lower than 99% confidence level ($P < 0/000$). The analysis of variances results (ANOVA), for the leaf area and weight of Ironwood species have been shown in table 2.

The results of Dunnett T3 test, exhibited leaf area (according to cm^2) decreasing, in parasitized branches compared with unparasitized and control trees branches of Ironwood species. The results are shown in Figure 1 (note: little T bars in top of each column, show the standard error values).

Table 2 - The mean comparison results of the leaf area and weight, using one-way ANOVA in branch groups ▪

	P	*F*▪
Leaf Area	0/000	45/669 **
Leaf Weight	0/000	26/989 **

▪The symbol of **States significant differences in 99% confidence level

Decreasing in leaf weight (according to gram) also, observed in parasitized compared with unparasitized and control trees branches of Ironwood species. The Tukey HSD test results is prepared in Figure 2.

Furthermore, the results of foliar analysis showed the presence of signifycant difference in amount of Potassium and Zinc in two hosts, and also Nitrogen and Manganese just in Ironwood species. But Phosphorus, Calcium, Magnesium and Sodium didn't reveal any significant difference among leaves of branches groups. The one-way ANOVA results for mineral nutrients of the two hosts have been prepared in table 3. Results of eight elements concentration in leaves of parasitized and unparasitized branches and control trees of the two hosts species

(Hornbeam and Ironwood species) are shown either in Figure 3.

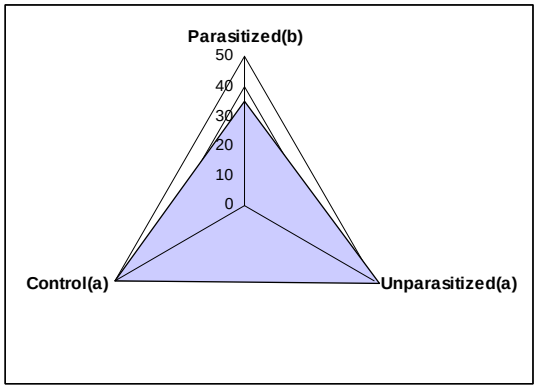

Figure 1 - The results of mean comparison of the leaf area in parasitized and unparasitized branches with control tree of *Parrotia persica* (Dunnett T3 test)

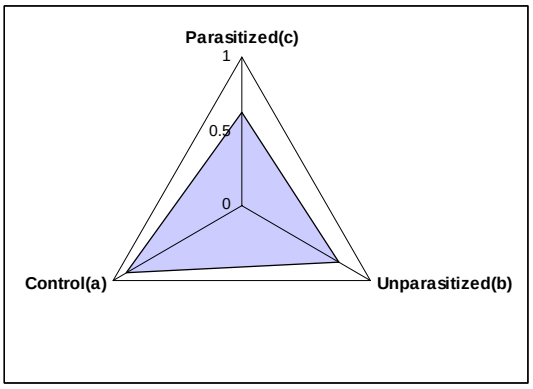

Figure 2 - The results of mean value comparison of the leaf area in parasitized and unparasitized branches with control tree of *Parrotia persica* (Tukey HSD test).

Table 3 - ANOVA results of mineral nutrients based on post-hoc mean comparison

Mineral elements	*Parrotia persica*		*Carpinus betulus*	
	F	**Sig.**	**F**	**Sig.**
N	7/667 **	0/007	0/283 **ns**	0/759
P	0/470 **ns**	0/636	0/703 **ns**	0/514
K	7/996 **	0/006	4/531 *	0/034
Ca	0/868 **ns**	0/445	0/799 **ns**	0/472
Mg	1/161 **ns**	0/346	1/083 **ns**	0/370
Zn	5/506 *	0/020	6/838 *	0/010
Mn	9/634 **	0/003	0/477 **ns**	0/632
Na	1/663 **ns**	0/230	0/331 **ns**	0/724

The symbols of ** and * state respectively the significant differences in 99% and 95% confidence level and also **ns** sign, shows not significant differences between groups

As shown in Figure 3, Nitrogen concentration was significant only in Ironwood tree species. The level of this element in parasitized branches was lower than the control tree and unparasitized branches were in the middle. Potassium amount in leaves of parasitized branches is higher than control tree in two species. But unparasitized ones were in the middle too. The amount of Zinc as well, was higher in infected than control trees of two species. The amount of Manganese in infected Ironwood trees was higher than control trees. Other nutrients have no significant difference among groups.

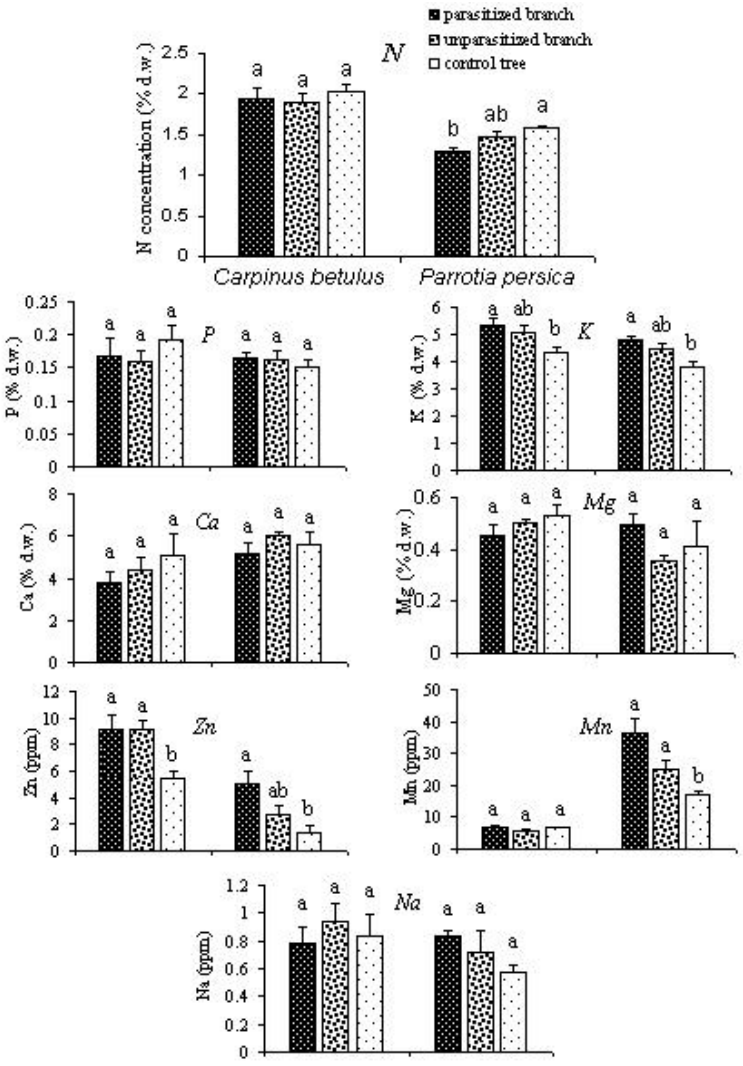

Figure 3 - The nutrient content results in leaves of parasitized and unparasitized branches and control trees of Hornbeam and Ironwood species

Discussion

Although mistletoe is a parasite and as such dependent on the host plant for nutrients and water, its does not rely on it for carbon dioxide. Since mistletoe produce green, chlorophyll-containing leaves, it can perform its own photosynthesis. European mistletoe is an aerial hemiparasitic plant, absorbing water and mineral nutrients from the host xylem and with either CO_2 fixation, is able to manufacture its necessary carbon compounds in photosynthesis process (TENNAKOON and Pate, 1996; HEIDE-JØRGENSEN, 2004; ZUBER 2004).

V. album similar to many other parasitic plants has a high transpiration rate and a low photosynthetic rate. The study of the nutrient contents of the xylem and seasonal measurements of the biomass and tissue nutrient contents of *Viscum* and *Pinus* have led to the hypothesis that the high rate of transpiration may be necessary for the parasites to take up sufficient nitrogen from the xylem of the host. Nitrogen is an important element for the production of biomass (ZUBER, 2004). Other studies of growth rate and accumulation of nutrients like N, P, K and Ca as well as values for carbon isotope ratios of mistletoe tissue further support the hypothesis that the higher transpiration rates of mistletoes represent a nitrogen gathering mechanism. Nitrogen potentially is the nutrient most limiting to mistletoe growth. Generally the solutes in the xylem sap of the host reach the mistletoe through the transpiration stream and according to this, the amount of nutrients like N, K, P, Ca and Mg are considerably higher in *Viscum* than in the host, specially when compared to infected host branches (ZUBER, 2004). The reason is that the hydrostatic pressure in the cells of the two parts of haustorial connection (between host and parasite) is in favor of the parasite and hence water with dissolved nutrients will always flow from the host to the parasite and not in the opposite direction (HEIDE-JØRGENSEN, 2004).

After the mistletoe infects host branches, the changes in host leaf area, leaf number, reduction in growth performance and biomass reflect the physiological and metabolic perturbation induced by the parasite (KARUNAICHAMY *et al.*, 1999). Competition for water, inorganic ions and metabolites is the simplest explanation for loss in host production and consequently area and weight of leaves (see Figure 1 and 2.)

Anyhow, the cause of decreasing and increasing in amount of some elements in host trees, will just distinguish by precise observation on the role of these elements in this study. Based on explanations of MARSCHNER (1995), high nitrogen supply increases the severity of infection by parasites and there is often a positive correlation between nitrogen application and pests attack. This function is because of the important role of nitrogen element in the photosynthesis process, and since the mistletoe parasites have no real root in soil and can not absorb mineral nitrogen directly from soil, embed compulsorily the haustoria in host xylem to uptake it (ZUBER, 2004; WATSON, 2001). The host plant also is not able to quickly absorb and compensate nitrogen supply; consequently we perceive the decreasing in amount of nitrogen in parasitized branches of *Parrotia persica*.

In contrast to nitrogen, high concentrations of potassium increase the resistance of host plants to both parasites attack and difficult environmental conditions (MARSCHNER, 1995). It would be inferred that confronting with the parasite, the host plant absorbs higher amount of potassium from the soil; as we clearly see the higher concentration of the element in parasitized branches of two host species (Figure 3).

Based on declarations of MARSCHNER (1995), micronutrients can also affect plants resistance indirectly. In deficient plants not only might the defense mechanism be impaired but often plants also become a more suitable feeding substrate. On the whole, micronutrient deficient plants suffered much more attack than micronutrient-deficient plants (MARSCHNER, 1995). As a result of these statements may be deduced the same inference about zinc and manganese that was done about potassium element. It means, the host plant upon being pressed and feeling danger from parasites attack, changes its metabolism toward absorbing more amounts of micronutrient from the soil if would be accessible.

Transfer of nutrients from the host to the parasites weakens the host to same degree. Normally, a parasite attack will not cause the death of the host, since the parasite at the same time will choose its foundation for existence. Generally, an attack of *Viscum* will result in reduced growth and fewer flowers and fruits on the twigs carrying the mistletoe (HEIDE-JØRGENSEN, 2004). However, the interaction between higher plants and parasites and pests are complex, and give a short outline of the role of mineral nutrients in these interactions requires considerable researches. Whereas the study has performed on host branches with one adult mistletoe and diameter of 7-10 cm, we suggest conducting similar studies on different severities of mistletoe infection and varied hosts in many times to monitor the effects of mistletoe on mineral nutrients better.

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