Seasonal variations of sulphur, phosphorus and magnesium in the leaves and current-year twigs of hemiparasitic mistletoe *Loranthus europaeus* Jacq. and its host *Quercus pubescens* Willd.

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**Abstract**


The objectives of this research were to investigate nutrient seasonal dynamics in the hemiparasitic mistletoe *Loranthus europaeus* von Jacquin and its host *Quercus pubescens* Willdenow, and to evaluate nutrient relationships between mistletoe and its host. For these purposes, S, P and Mg concentrations in the leaves and current-year twigs were analysed 12 times during the growing season. We found that the studied nutrients were not retrieved from hemiparasitic mistletoe leaves prior to abscission, contrary to its host. The seasonal dynamics of S, P, and Mg in *L. europaeus* and *Q. pubescens* leaves differed from each other while in current-year twigs the dynamics was similar in both species. In general, nutrient concentrations in the leaves and current-year twigs were higher in mistletoe compared with its host. But the mistletoe to host nutrient ratios varied greatly during the growing season, especially in leaves, mostly during leaf expansion and senescence. Thus, studies investigating nutrient relationships between mistletoe and its host should be realized during the period of leaf maturity.

**Keywords**: active transport; leaf development; mistletoe-host interaction; nutrient relationships; nutrient remobilization; passive transport

Mistletoes are perennial flowering hemiparasitic or holoparasitic plants attached to the branches of trees and shrubs which affect host viability by withdrawing water and mineral nutrients (Watson 2001; Garkoti et al. 2002; Glatzel, Geils 2009), and also significant amounts of carbohydrates (Escher et al. 2004). Whereas holoparasites import minerals via both the xylem and the phloem, hemiparasites get most minerals from the host xylem sap, diverting it from the host by various ways of xylem connection in the haustorium (Glatzel 1983; Bell, Adams 2011). As a consequence, most hemiparasites have very few phloem elements in the haustorium, and they terminate well before the host-mistletoe interface (Glatzel 1983). However, despite the long history of independent observations on mistletoe (Kamerling 1910; Senn 1913), the precise mechanism of water and mineral trans-
port from the host to mistletoe is not yet fully understood (Panvini, Eickmeier 1993; Mathiasen et al. 2008). The question whether nutrient uptake in mistletoe is only passive or partly passive and partly active has not been conclusively resolved yet (Press et al. 1990; Panvini, Eickmeier 1993; Bowie, Ward 2004; Glatzel, Geils 2009). The partly active nutrient uptake theory suggests that nutrient and water acquisition may not be tightly coupled, that some mechanism exists in the haustorium to facilitate active nutrient loading, and that the host phloem also contributes, even if indirectly, to the nutrient status of the parasite (Panvini, Eickmeier 1993; Cocoletzi et al. 2016). Moreover, observations that the enrichment with some nutrients is higher than with others have been interpreted as an indicator of the selective uptake of ions via the haustorium (Atsatt 1983; Lamont 1983). In general, hemiparasites exhibit higher nutrient concentrations compared with their host plants, ranging from 1.2 times higher for zinc to 20 times higher for K (Lamont, Southall 1982; Hollinger 1983; Press et al. 1990; Gebauer et al. 2012).

Experiments with mistletoe growing on trees are difficult as it grows in the crown and is quite inaccessible for sampling (Yang et al. 2017). Due to this fact, nutrient relationships between mistletoe and its host are usually based only on one sampling date (Glatzel 1983; Schulze et al. 1984; Bowie, Ward 2004; Türe et al. 2010). Although fully expanded and hardened leaves were used for nutrient analysis in these studies as recommended by Cornelissen et al. (2003), the nutrient concentration in mistletoe and its host could vary even in the adult leaves between the dates (Escher et al. 2004; Gebauer et al. 2012). In addition to the nutrient dynamics within the main season, the nutrient remobilization during leaf senescence is even more poorly understood. Nutrient correlations within and between mistletoe and its host represent other important information which has not yet been understood sufficiently but which can provide an insight into the physiology of coexistence of these two organisms. It was proposed that the concentration of a particular nutrient in mistletoe is often correlated with the concentration of the same nutrient in the host (Panvini, Eickmeier 1993; Gebauer et al. 2012). The exact concentrations of nutrients and their balance are likely regulated by an interplay between endogenous and environmental factors (Bongard-Pierce et al. 1996). Thus, information about nutrient dynamics in the hemiparasitic mistletoe and its host during the growing season could improve our understanding of the mistletoe-host relationship. Better knowledge of the mistletoe-host interaction can also be utilized to improve the management of infested forest plantations for resource production as well as for the conservation of biodiversity.

The objectives of this research were (i) to investigate S, P and Mg dynamics in the hemiparasitic mistletoe Loranthus europaeus von Jacquin and its host Quercus pubescens Wildenow during one growing season, (ii) to evaluate S, P and Mg relationships between L. europaeus and its host Q. pubescens during one growing season, (iii) to evaluate nutrient correlations within and between L. europaeus and its host Q. pubescens. This study follows up previous studies on leaf development and the nutrient dynamics of N, K and Ca in L. europaeus and Q. pubescens (Gebauer et al. 2012) and on the comparison of transpiration and stomatal conductance in L. europaeus and Q. pubescens (Urban et al. 2012).

MATERIAL AND METHODS

Experimental plot. The experimental plot was situated on the Pouzdraný Steppe (Czech Republic; 48°56′52.46″N and 16°38′41.28″E; 278–295 m a.s.l.). This site is heavily infested with the hemiparasitic mistletoe L. europaeus growing on several oak species, Quercus petraea (Mattuschka) Lieblein, Q. pubescens and Quercus robur Linnaeus, which are growing solitarily. The mean annual precipitation at the site varies from 450 to 500 mm and the mean annual temperature is 9.5°C. The average height of adult oak trees is only 6 m due to frequent drought periods during the growing season (Urban et al. 2012).

Sample collection. We sampled leaves and current-year twigs from the same trees used in our previous study (Gebauer et al. 2012). Samples of Q. pubescens and hemiparasitic L. europaeus twigs with attached leaves were collected 12 times during the growing season in 2009 (from spring to autumn; sampling days: 14.4., 20.4., 27.4., 11.5., 18.5., 1.6., 18.6., 20.7., 26.8., 23.9., 21.10. and 10.11.). All samples were taken randomly from the sun-exposed parts of the crowns of eight oak trees infested by the hemiparasite (one male hemiparasite per oak tree was sampled). Oak samples were taken from non-infested branches. At least three twigs were taken from each oak tree and mistletoe. The samples were divided into leaves and current-year twigs. Ten oak leaves and 20 mistletoe leaves were randomly chosen and dried at 80°C for 2 days to
analyse mean leaf dry mass (Gebauer et al. 2012). Then, immediately after each sampling date, the plant material from all oak trees and mistletoes was mixed (separately leaves and current-year twigs) and dried at 80°C for 2 days. As a result, we obtained one composite sample taken from eight trees (n = 1) of twigs and leaves of oak and mistletoe for each sampling date. We took enough plant material to have at least 20 g of each part needed for nutrient analyses.

Nutrient analysis. The dry samples of leaves and current-year twigs were sent to an accredited laboratory (Laboratoř Morava, s.r.o., Studénka, Czech Republic) for S, P and Mg concentration analysis. P and Mg were analysed by atomic absorption spectrophotometry and S by gravimetric analysis. The results were obtained in terms of gram of nutrient per kilogram of dry mass (nutrient\text{mass}). We also studied the seasonal dynamics of nutrient concentration per mean leaf in both species. This was particularly important to evaluate nutrient remobilization. Mean leaf dry mass (DM) and nutrient concentration per dry mass (nutrient\text{mass}) were needed to calculate the nutrient concentration per mean leaf (nutrient\text{leaf}) (Eq. 1):

\[
\text{nutrient}_{\text{leaf}} = \frac{\text{DM} \times \text{nutrient}_{\text{mass}}}{1,000,000} \quad \text{(mg)}
\]  

Statistical analysis. Nutrient analysis of leaves and current-year twigs was done on composite samples taken from eight oak trees and eight mistletoe plants (n = 1). We used Pearson’s correlation coefficient to analyse the relationships during studied period: (i) between nutrients within the host and within the hemiparasite, (ii) between the host and hemiparasite. Moreover, Pearson’s correlation coefficient was used to evaluate (iii) the relationship between nutrient concentrations in host and hemiparasite and the day of the year. Separate correlation matrices with correlation coefficients and scatterplots were set up for leaves and current-year twigs. We also included a significance test for the correlation coefficients. Statistical analyses were carried out using the R statistical program (Version 3.1.3, 2015).

RESULTS

Seasonal nutrient dynamics per mean leaf

For Loranthus von Jacquin, S\text{leaf} reached a maximum at the end of August and then S\text{leaf} sharply decreased (by 56%) till the end of the growing season (Fig. 1a). For Quercus, P\text{leaf} reached a maximum at the end of October and then P\text{leaf} decreased by 20% till the end of the growing season (Fig. 1b). For Quercus, P\text{leaf} reached two maxima (at the end of April and at the end of July) (Fig. 1b) and then P\text{leaf} decreased by 80% till the end of the growing season. For Loranthus, Mg\text{leaf} increased steadily throughout the growing season (Fig. 1c). In contrast, Mg\text{leaf} in Quercus leaves reached a maximum at the end of August and then decreased by 15% till the end of the growing season (Fig. 1c).

Seasonal nutrient dynamics per unit dry mass

In general, nutrient concentration per unit dry mass was higher in the leaves than in the current-year twigs for both species (Fig. 2). The only ex-
ception was P\textsubscript{mass} of \textit{Loranthus} (Figs 2c, d). The seasonal dynamics of S\textsubscript{mass}, P\textsubscript{mass} and Mg\textsubscript{mass} in the leaves of \textit{Loranthus} and \textit{Quercus} differed from each other. Three peaks of high S\textsubscript{mass} in leaves were observed during the growing season for both species (Fig. 2a). The first occurred in April, the second in June, and the third in August. The lowest concentrations of S\textsubscript{mass} in leaves occurred in the middle of May and in July for both species. On the other hand, P\textsubscript{mass} of \textit{Loranthus} and \textit{Quercus} leaves decreased till the end of July and then fluctuated around the same value until the end of the growing season (Fig. 2c). Another situation was observed for Mg\textsubscript{mass}. For \textit{Loranthus} leaves, Mg\textsubscript{mass} first increased till the end of June, then it decreased sharply (by 30%) in July, after which it fluctuated around the same value till the end of the growing season (Fig. 2e). On the contrary, Mg\textsubscript{mass} of \textit{Quercus} leaves fluctuated around the same value during the growing season (Fig. 2e).

Conversely to the seasonal nutrient dynamics of \textit{Loranthus} and \textit{Quercus} leaves, the seasonal nutrient dynamics of current-year twigs was similar for both species (Figs 2b, d, f). S\textsubscript{mass}, P\textsubscript{mass} and Mg\textsubscript{mass} of current-year \textit{Loranthus} and \textit{Quercus} twigs were highest at the beginning of the season, then gradually decreased until the middle/end of June, after which it fluctuated around the same value until the end of the growing season. The only exception was S\textsubscript{mass} of current-year \textit{Loranthus} twigs, which peaked in the middle of June (Fig. 2b).

\section*{Mistletoe to host nutrient ratio}

In general, the mistletoe to host nutrient ratios (L/Q) for S\textsubscript{mass}, P\textsubscript{mass} and Mg\textsubscript{mass} were higher in the \textit{Loranthus} leaves and current-year twigs than in \textit{Quercus} (L/Q > 1) (Fig. 3). The exceptions were

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig2.pdf}
\caption{Seasonal dynamics of nutrient concentrations per unit dry mass in leaves: S (a), P (c), Mg (e) and current-year twigs: S (b), P (d), Mg (f) of \textit{Quercus} Linnaeus and \textit{Loranthus} von Jacquin during the 2009 growing season (n = 1)}
\end{figure}
higher S\textsubscript{mass} in Quercus leaves from the middle of June till the middle of July, higher Mg\textsubscript{mass} in Quercus leaves at the beginning of the growing season and higher Mg\textsubscript{mass} in current-year Quercus twigs for a short period at the end of July (Fig. 3). There were large differences in mistletoe to host nutrient ratios (L/Q ratios) in leaves during spring and autumn seasons (Fig. 3). On the other hand, mistletoe to host nutrient ratios in leaves were almost similar during the summer season. For current-year twigs, the mistletoe to host nutrient ratios (L/Q) were higher for P\textsubscript{mass}, moderate for S\textsubscript{mass} and the lowest for Mg\textsubscript{mass} during the growing season (Fig. 3).

**Correlation within and between species**

Strong correlations between the day of the year and nutrient concentrations in the current-year twigs were found for both species (Fig. 4b). On the other hand, only P\textsubscript{mass} in leaves of both species was correlated with the day of the year (Fig. 4a). Nutrients in current-year twigs within both species were strongly correlated with each other (the only exception was the correlation between S\textsubscript{mass} and P\textsubscript{mass} in Loranthus) (Fig. 4b). In contrast, there was no correlation between nutrients in leaves within both species (Fig. 4a). There were only a few correlations between both species in leaves and current-year twigs. Strong correlations between species were found for P\textsubscript{mass} in leaves as well as in current-year twigs (Figs 4a, b). Two other correlations were observed between species in current-year twigs (Fig. 4b).

**DISCUSSION**

**Seasonal nutrient dynamics per mean leaf and per dry mass**

Leaf development can be divided into three major periods: leaf expansion, leaf maturity, and leaf senescence (Kozlowski 1971). In general, leaf expansion in deciduous angiosperms (i.e. Quercus sp.) is quite rapid and the final leaf area is achieved within a few days to a few weeks (Kozlowski 1971). However, the growth of Loranthus leaves rather resembles that of evergreen angiosperms, as leaf expansion in Loranthus took more than four months (Kozlowski 1971; Gebauer et al. 2012). The slow development of the Loranthus leaf is in agreement with our observation of steadily increasing S\textsubscript{leaf}, P\textsubscript{leaf} and Mg\textsubscript{leaf} during the growing season.
season. Moreover, it also corresponds with findings that even essential nutrients are not retrieved from hemiparasitic mistletoe leaves prior to abscission (PATE 1995). Only a small decrease of \( P_{\text{leaf}} \) was observed in \textit{Loranthus} at the end of the growing season. On the other hand, nutrient remobilization was observed in \textit{Quercus} leaves, although the start of remobilization differed for each nutrient. Several authors have reported that decreases in leaf nutrient concentrations correspond with leaf senescence (MOORE 1966; KOZLOWSKI 1971). However, differences in the timing of nutrient concentration decreases in \textit{Quercus} leaves (i.e. from the end of April till the end of August) correspond more with the finding that nutrient remobilization is at least partially independent of leaf senescence (MAILLARD et al. 2015). Different remobilization rates of the studied nutrients observed in \textit{Quercus} leaves indicate their mobility in the phloem (PANVINI, EICKMEIER 1993). Although the mobility of P, S, and Mg in the phloem is considered to be high (MENGEL, KIRKBY 2001; WHITE 2012), the remobilization of Mg_{leaf} was low (only 15%) compared to S_{leaf} (56%) or P_{leaf} (80%).

Considering the nutrient concentration per dry mass, P_{mass} in \textit{Loranthus} leaves was in the range reported for hemiparasitic mistletoe (LAMONT 1983; BANNISTER et al. 2002). However, concentrations of Mg_{mass} and S_{mass} in \textit{Loranthus} leaves were lower than those reported for other mistletoes (LAMONT 1983; TÜRE et al. 2010). Interestingly, the seasonal dynamics of S_{mass}, P_{mass} and Mg_{mass} in \textit{Loranthus} and \textit{Quercus} leaves differed from each other, contrary to nutrient dynamics in current-year twigs. There could be several reasons for such behaviour. For example, it could be connected with differences in S, P and Mg phloem mobility, their usage during leaf development, response to stress, ability to be leached from leaf during rain or most probably due to a combination of several factors. To solve this question a detailed research is needed.

**Mistletoe to host nutrient ratio**

The mistletoe to host nutrient ratio is often used to indicate whether mistletoes accumulate nutrients in excess of those of their hosts (GLATZEL 1983; SCHULZE et al. 1984; PANVINI, EICKMEIER 1993). It was proposed that the higher concentrations of nutrients observed in hemiparasitic mistletoe were a consequence of higher transpiration rates in mistletoe combined with the absence of a retranlocation system between mistletoe and its host (GLATZEL 1983; LAMONT 1983; PANVINI, EICKMEIER 1993). In our study, a general trend of higher nutrient concentrations in mistletoe compared with its host was confirmed. Nevertheless, S_{mass} or Mg_{mass} in leaves were higher for a short period during spring (i.e. during the period of leaf expansion) in the host than in mistletoe. TÜRE et al. (2010) found also higher concentrations of Ca, Mg or Fe in the host than in mistletoe leaves.

Observations that the enrichment of mistletoe with some nutrients is higher than with others have been interpreted as indicating the selective uptake of ions via the haustorium (ATSATT 1983; LAMONT 1983). The reason behind is that if the uptake is passive via the transpiration stream, the mistletoe to host nutrient ratio for each nutrient should be the same, only an overall nutrient concentration could be different due to differences in relative rates of transpiration in mistletoe and the host. In our study, the mistletoe to host nutrient ratios varied greatly during the growing season, especially in leaves, more during the leaf expansion in spring and during the leaf senescence in autumn. However, in agreement with the passive nutrient uptake theory, the mistletoe to host nutrient ratios were stable and similar for all studied nutrients in summer. This emphasizes a recommendation by CORNELISSEN et al. (2003) that the nutrient analysis for species comparison should be done on fully expanded and mature leaves. Our data indicate that concentrations of nutrients in the mistletoe vary both up and down, but so far we have not been able to identify a mechanism of this variability (i.e. to what extent the xylem and possibly the phloem contribute to nutrient translocation to and from the mistletoe). The important point within the mistletoe to host nutrient ratio hypothesis is that it does not take into account the recycling of nutrients that can take place in the host via the phloem, which is restricted in mistletoe (GLATZEL 1983). Although no direct connection between the phloem sieve tube elements of the hemiparasite and its host was observed, the possibility of a symplastic pathway cannot be ruled out (PATE 1995; BELL, ADAMS 2011). In a recent study, phloem parenchyma cells of the hemiparasitic mistletoe \textit{Psittacanthus schiedeanus} (von Schlechtendal & von Chamisso) G. Don developed half-plasmodesmata in their cell walls, if they were in contact with parenchyma cells of its host \textit{Liquidambar styraciflua} Linnaeus (COCOLETTI et al. 2016). Thus, the function of the haustorium of hemiparasites could be more complex than that of an organ that simply channels solutes from the host xylem to the hemiparasite (LAMBERS et al. 2008).
To reveal the phloem connection between a host and its hemiparasite, translocation experiments using fluorescent probes or isotopic labelling should be performed. These methods were used successfully to study phloem connections between several species of the holoparasite *Cuscuta* sp. and its compatible host (Birschwilks et al. 2006).

**Nutrient correlations within and between species**

It was proposed that the best indicator for predicting the concentration of a particular nutrient in mistletoe is often the concentration of the same nutrient in the host, especially in the leaves and current-year twigs (Panvini, Eickmeier 1993; Glatzel, Geils 2009; Gebauer et al. 2012). However, in our study, this finding was valid only for P. Although nutrients such as N, K, and Ca were found to be correlated with each other within the leaves within as well as between the hemiparasite and its host (Gebauer et al. 2012), no such correlations were observed in this study. Only concentrations of nutrients in current-year twigs were correlated with each other within both species, and they were also dependent on the day of the year.

**References**


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