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ANNUAL VARIATIONS OF THE OCCURRENCE OF LICHEN PHENOLICS FROM *EVERNIA PRUNASTRI* IN THE XYLEM SAP OF *QUERCUS ROTUNDIFOLIA*

Jährliche Schwankungen des Auftretens von Flechtenphenolen von *Evernia prunastri* im Xylemsaft von *Quercus rotundifolia*

by

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Key words: *Evernia prunastri,* evernic acid, *Quercus rotundifolia,* usnic acid.

Schlagwörter: *Evernia prunastri,* Evernsäuren, *Quercus rotundifolia,* Usninsäure.

Summary: Epiphytic lichens can have damaging effects on trees. They penetrate to different degree into phytophore tissues. *Evernia prunastri* hyphae progress into xylem vessels of *Fagus sylvatica, Quercus pyrenaica* and *Betula alba.* This would provide a structural basis for a chemical interaction between lichen metabolites and metabolic processes of the phytophore. Oak branches supporting the epiphytic lichen *E. prunastri* contain both evernic and everninic acids which are acropetally transported via xylem to reach the leaves. In this work, we appraised the annual variations of lichen phenolics in the xylem sap of *Q. rotundifolia* branches by TLC and HPLC analysis and we remarked the absence of lichen phenolics in branches without epiphytic lichens. These phenolics certainly produce defoliation and inhibit the development of foliar buds.

Zusammenfassung: Epiphytische Flechten können schädigende Effekte auf Bäume ausüben. Sie dringen in unterschiedlichem Maße in das Gewebe der Phorophyten ein. Die Hyphen von *Evernia prunastri* erreichen die Xylemgefäße von *Fagus*

sylvatica, Quercus pyrenaica und *Betula alba*. Dies würde die strukturelle Basis für eine chemische Interaktion zwischen Flechten-Metaboliten und den metabolischen Prozessen des Phorophyten bilden. Eichenäste, die die epiphytische Flechte *E. prunastri* tragen, enthalten Evern- und Everninsäure, welche akropetal über das Xylem bis zu den Blättern transportiert werden. In der vorliegenden Arbeit schätzten wir die jährlichen Schwankungen der Flechtenphenole im Xylemsaft der Äste von *Quercus rotundifolia* mit Hilfe von TLC und HPLC ab und wir stellten auch die Abwesenheit von Flechtenphenolen in Ästen ohne epiphytische Flechten fest. Diese Phenole führen zum Blattfall und sie hemmen das Wachstum der Blattknospen.

Introduction

Upto day the relationship between lichens and substrate stay unclear at all. There may be extensive penetration of the rhizines through the cork, cortex and cambium until the living wood (Ascaso et al. 1980). These hyphae readily tear off areas of both phloem and xylem and they disperse either individually or in small groups towards the vessels (Ascaso 1985). Oak branches supporting the epiphytic lichen *Evernia prunastri* (L.) Ach. and *Ramalina calicaris* (L.) Fr. contain evernic and everninic acids, which are acropetally translocated, via xylem to reach the leaves (Avalos et al. 1986). This mechanism was proposed by Ozenda and Clauzade (1970) for both usnic and sekikaic acids produced by *Ramalina tayloriana* Zahlbr.

The purpuse of this work is to find the occurrence of the lichen phenolics from *Evernia prunastri* in the xylem sap of *Quercus rotundifolia* and their variation from January to September.

Materials and Methods

Primary, secondary and tertiary branches of *Quercus rotundifolia,* covered by *Evernia prunastri,* were collected in El Pardo de Madrid (Spain), and then were put in tubes that were sealed with paraffin at the top, and transported to the laboratory for analysis; as control primary, secondary and tertiary branches of *Q. rotundifolia* without epiphytic lichens were used.

Portions of about 0.175 g weight of both apical and basal ends of branches were cut, placed with the morphological upper end at basal position in centrifuge tubes and spun at 5000 x g for 15 min (modified from ref. Avalos el al. 1986) to extract the xylem sap. Residues were dissolved in acetone for analyis by TLC, air-dried and redissolved in acetonitrile for analysis by HPLC.

TLC were devoloped on Merk Silicagel G-60, using benzene:dioxane:glacial acetic acid (90:25:4) as solvent (Ramault 1963, a) and the spot localized by fluorescence emission after exciting with light of 366 nm wavelenght. Also, they were sprayed with 10% H_2SO_4 and heated at 100° C for 1 hour.

Usnic and evernic acids, and atranorin (Sigma) were used as standards.

Rf values were taken as 0.74 for evernic acid, 0.92 for usnic acid and 0.94 for atranorin.HPLC analysis were performed according to LEGAZ and VICENTE (1983) by using a varian 5000 liquid chromatograph equipped with a vista CDS 401 Computer. Chromatographic conditions were as follows: reverse phase column 200 x 4 mm i.d., paked with micropak MCH-10; mobile phase, acetic acid:water (2:98) acetonitrile (1:4 v/v); flow rate, 0.7 ml min^{-1}; pressure, 66 atm.; temperture, 25° C, absorbance range, 0,002; detector, UV at 254 nm.

Results

Analysis by TLC of the *Quercus rotundifolia* xylem sap indicates the existence of the main phenolic from *Evernia prunastri*, that develop colour with sulfuric acid after heating. Rf value was taken as 0.71 that is the same as evernic acid standard.

Monthly analyses by HPLC, from January to September, confirm the occurrence of evernic and usnic acids in the xylem sap of *Quercus rotundifolia*, the concentration of which in primary brances was always higher than that found in secondary and tertiary branches (Fig. 1 & 2). Evernic acid was detected in the xylem sap of *Q. rotundifolia* for all the months assayed (Fig. 1), but usnic acid only accumulated from February (Fig. 2). In petioles, evernic acid was the sole phenolic observed (28.3 $\mu g.g^{-1}$ dry weight) but only in March. In buds, evernic acid was detected in secondary buds in April (31.4 $\mu g.g^{-1}$ dry weight) and in primary buds in May (14.5 $\mu g.g^{-1}$ dry weight), but usnic acid was detected only on June (Table 1). However, leaf and nervure extracts do not contain evernic nor usnic acids.

Discussion

Several lichens can inhibit bud develepment in tea plants (ASAHINA el al. 1952). FOLLMANN and NAKAGAWA (1963) show the allelopathic action of thallus extracts from *Sticta weigelii* on both germination and growht in pea. Defoliation of beech and live-oak trees having lichens has been reported (ESTÉVEZ et al. 1982), although it is not clear whether it is a consequence of accelerated senescence or an inhibition of new leaf formation. The acropetal translocation of evernic acid in the xylem of oak branches exists (AVALOS et al. 1986). Oak shrubs which support epiphytes differentiate about 20% less buds in their primary branches and about 40% less buds in their secondary ones than shrubs without epiphytes (LEGAZ el al. 1988).

These results, that show the occurrence of evernic and usnic acids and their monthly variations from January to September in the xylem sap of *Quercus rotundifolia*, confirm that there was an acropetal traslocation not only for evernic acid, but also for usnic acid. Their occurrence in xylem sap, petioles

and buds may be related with defoliation of *Quercus rotundifolia* and the least appearance of buds.

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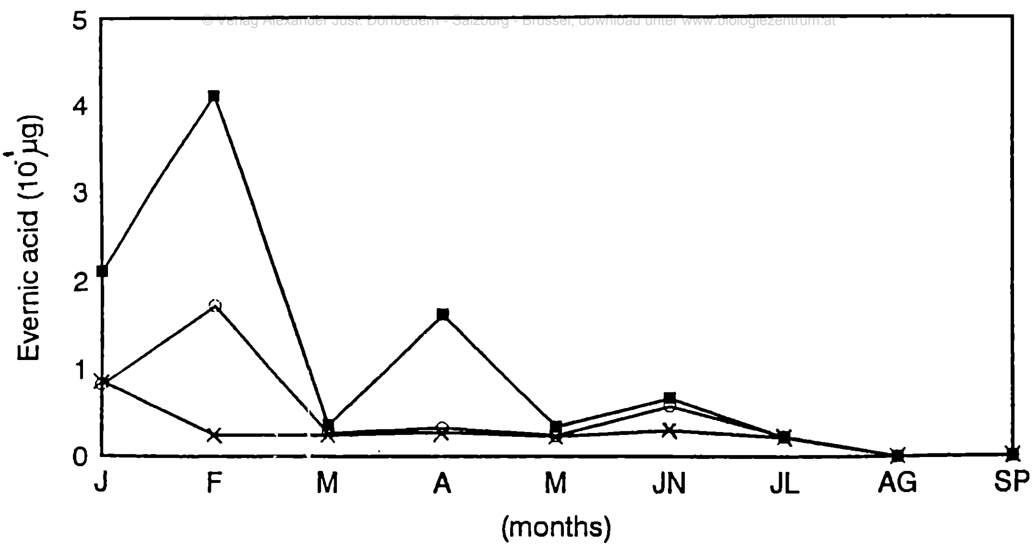

Fig. 1: Monthly variation of evernic acid in primary (■), secondary (O) and tertiary (X) branches of *Quercus rotundifolia* with epiphytic lichens, from January to September.

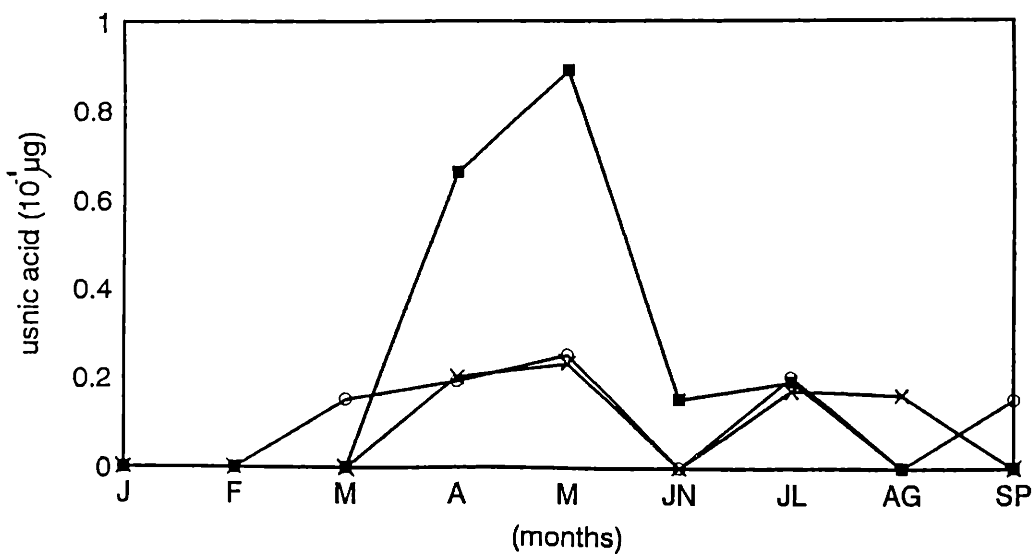

Fig. 2: Monthly variation of usnic acicd in primary (■) secondary (O) and tertiary (X) branches of *Quercus rotundifolia* with epiphytic lichens, from January to September.

Table 1: The occurrence of usnic acid in primary secondary and tertiary branche buds of *Quercus rotundifolia* on June.

	Weight of buds (mg.)	Usnic acid (µg.)	Usnic acid ($\mu g.g^{-1}$ dry weight)
Primary branche bud	9	$3.20\ 10^{-2}$	3 .6
Secondry branche bud	9.1	$3.83\ 10^{-2}$	4 .2
Tertiary branche bud	11. 6	$3.81\ 10^{-2}$	3 .3