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EFFECTS OF LICHENS PHENOLICS ON DEFOLIATION OF *QUERCUS ROTUNDIFOLIA* LAM.

Der Einfluß von Flechtenphenolen auf den Laubfall von *Quercus rotundifolia* LAM.

by

Khalid BOUAID & Carlos VICENTE

Key words: Chlorophyll, lichen, phenolics.
Schlagwörter: Chlorophyll, Flechten, Phenole.

Summary: Epiphytic lichens play a secondary role as pathological organisms against their phytophores on which they grow and, thus, it is a common practice to destroy corticolous lichens growing on fruit trees with fungicides. Branches of *Quercus pyrenaica* WILLD. and *Quercus rotundifolia* LAM. supporting a heavy population of epiphytic lichens are often largely defoliated. Medulary hyphae can penetrate into host tissues by both mechanical and enzymatic action, to reach xylem vessels. So far this can be due to a mechanism through which lichens inject metabolic inhibitors that, after their translocation in the xylem sap, reach leaves and produce abscision. Chloroplasts isolated from detached leaves of *Quercus rotundifolia* were incubated with lichen substances isolated from *Evernia prunastri* thallus that produced inhibition of the Hill reaction. In this report, we demonstrate that the incubation with lichen substances unhangs magnesium ions from chlorophyll and produces the liberation of phaeophytins. On the other hand, those lichen substances diminished the amount of chloroplastic manganese through a chelation process. These effects cause loss of photosynthetic activity because the Hill reaction is restored upon addition of exogenous magnesium and manganese to the chloroplasts.

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Zusammenfassung: Epiphytische Flechten spielen sekundär eine Rolle als pathogene Organismen gegenüber den Trägerpflanzen, auf denen sie wachsen. Daher ist es eine übliche Praxis, rindenbewohnende Flechten, die Obstbäume besiedeln, mit Fungiziden zu vernichten. Äste von *Quercus pyrenaica* WILLD. und *Quercus rotundifolia* LAM. mit einer großen Population von epiphytischen Flechten sind oft weitgehend entlaubt. Hyphen der Markschicht dringen auf mechanischem und enzymatischem Weg in das Gewebe des Wirtes bis in das Xylem ein. Das kann einerseits auf einen Mechanismus zurückgeführt werden, bei dem Flechten metabolische Inhibitoren injizieren, die auf dem Weg über den Xylemtransport Blattfall bewirken. Isolierte Chloroplasten abgelöster Blätter von *Quercus rotundifolia* wurden in Flechtensubstanzen inkubiert, die aus Thalli von *Evernia prunastri* isoliert wurden und eine Hemmung der Hill Reaktion bewirkten. In diesem Bericht zeigen wir, daß eine Inkubation in Flechtensubstanzen eine Abkoppelung von Magnesium-Ionen vom Chlorophyll hervorruft und eine Freisetzung von Phaeophytinen bewirkt. Andererseits verringern diese Flechteninhaltsstoffe den Mangangehalt der Chloroplasten, weil sie als Chelatoren wirken. Dieser Effekt verringert die Photosyntheseaktivität, da die Hillreaktion vom Magnesium- und Mangangehalt in den Chloroplasten abhängig ist.

Introduction

Little is known about the effects of lichen thalli attached to higher plants. They can cause tree to defoliate (HALLE 1983). Through the work of SCHATZ et al. (1954), SCHATZ (1963) and VICENTE (1975), it is known that lichen substances deposited on fungae hyphae have a chelating effect through which epiphytic lichens may penetrate to different degree into phorophyte tissues the thallus receives inorganic cations from the substrate where they *Fagus sylvatica* (ESTÉVEZ et al. 1980) and *Quercus pyrenaica* (ASCASO et al. 1980). ORUS, ESTÉVEZ and VICENTE (1981) found that lichen substances, isolated from *Evernia prunastri* (L.) ACH. thalli, interfered with some live. (BRODO 1973), *Evernia prunastri* hyphae progress into xylem vessels interfering with physiological processes of *Quercus rotundifolia* LAM. foliage and then can inhibit photolytic activity through the chelation of the chloroplast manganese. The interaction between lichen and leaf metabolism has been studied in relation to the photolysis because of its dependence on manganese, but the interaction between lichen acids and chloroplast structure has been studied to an ultrastructural level.

Materials and Methods

Samples of *Evernia prunastri* thalli were superficialy washed off to remove cortical phenols. The extract, evaporated to dryness, contained evernic and D-usnic acids, and atranorin. Samples of this mixture were chromatographed on a thin layer of Silicagel G-60 using benzene: dioxane: glacial acetic acid (90:25:4) as solvent (RAMAULT 1963, a) and the spots localized by fluorescence emission after exciting with light of 366 nm wavelenght. Evernic and D-usnic

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acids, and atranorin (Sigma) were used as markers. Rf values were taken as 0.74 for evernic acid, 0.92 for usnic acid and 0.94 for atranorin. Phenolics were eluted with acetone and evaporated to dryness.

Young branches of *Quercus rotundifolia* were cut and removed at the same height level on the trunk, the zone of cutting being immersed in 1mM $NaHCO_3$ solution. There were four solutions, the first one was as control without usnic acid, but all the three other contained usnic acid 14µg/ml. At the second day we added 0.2 mM $MgCl_2$ in the third solution and 0.2 mM $MnCl_2$ in the fourth solution.

Chloroplasts were isolated by grinding to a fine powder with liquid nitrogen in 75 mM phosphate buffer, pH 6.9. The homogenate was filtered through a multilayer gauze and centrifuged at 7000 x g for 10 min at 0° C. The pellet was washed with 75 mM phosphate buffer and used as preparation of chloroplasts.

The Hill reaction was followed in mixtures containing, in a final volume of 9.0 ml, 1.0 ml of 0.52 mM DCPIP, 1.0 ml of lamellar suspension and 7.0 ml of 75 mM phosphate buffer, pH 6.9. The mixtures were illuminated, at 25° C, with a lamp 100W for 20 min. Units of Hill reaction were defined as the decrease of the absorbance at 600 nm. per µg of chlorophyll and minute.

Quantity of chlorophyll in *Q. rotundifolia* leaf with and without epiphytic lichens were estimated according to STRAIN et al. (1971).

Degradation of chlorophyll a and b was observed after their incubation with 0.1N HCl and 0.23 mg/ml usnic acid for 90 min. and their concentration in solution was estimated each 15 min.

Results

Measurement of chlorophyll a and b concentration from January to April showed that it was always higher in *Quercus rotundifolia* leaves without epiphytic lichens than those with epiphytic lichens (Fig.1), moreover the Hill reaction activity estimated by oxygen evolution, was influenced by epiphytic lichens (Fig. 2).

Isolated chlorophylls from *Quercus rotundifolia* leaves incubated with 0.1 N HCl and 0.23 mg/ml usnic acid in another solution, showed a progressive decrease in their concentration in solution, whereas chlorophyll control incubated in normal conditions maintained this concentration almost constant (Fig. 3).

Isolated chloroplasts from *Quercus rotundifolia* leaves of branches incubated 5 days in four solutions, three of them containing 14µg/ml of usnic acid in each one, showed a decrease in the Hill reaction, but one day after adding 0.2 mM $MgCl_2$ in the third solution and 0.2 mM $MnCl_2$ in the fourth solution, the Hill reaction activity increased, at the fourth day were marked a significant

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decrease in the Hill reaction for the second solution containing usnic acid only, but the Hill reaction maintained almost constant in the third and the fourth solution containing respectively 0.2 mM $MgCl_2$ and 0.2 mM $MnCl_2$ (Fig. 4).

Discussion

After treating *Q. rotundifolia* with a solution of extracts from *Evernia prunastri*, ORUS et al. (1981) noticed a reduction of photolytic capacity and Mn^{2+} content of chloroplasts. INOUE et al. (1983) confirmed these results with *Q. mongolia* treated with usnic acid. RAPSCH and ASCASO (1985) found that spinach leaves, in the presence of evernic acid, showed chlorosis and an altered internal organisation of chloroplasts from the spongy parenchyma. The chelating action of lichen substances is responsible for certain regulation mechanism in lichens, controlling divalent cation-dependent processes (VICENTE and ESTÉVEZ 1976; ESTÉVEZ and VICENTE 1978). Manganese is able to transfer electrons from water to P-680 (CHENIAE and MARTIN 1967). ASCASO and RAPSCH (1986) found a noticeable reduction of total chlorophyll, chlorophylls a and b, possibly due a decrease of Mg^{2+}, which stabilizes chlorophyll.

After treating chlorophyll from *Q. rotundifolia* leaves with 0.1 N HCl and with 0.23 mg/ml usnic acid, we remarked a drastic loss of chlorophylls a and b (Fig. 3). On the other hand after incubating *Q. rotundifolia* branches with usnic acid, we remarked a reduction of Hill reaction activity but this activity was restored one day after adding $MgCl_2$ and $MnCl_2$ (Fig. 4).

Usnic acid acts in a similar way to HCl and unhangs magnesium ions from chlorophyll and produces the liberation of phaeophytins. On the other hand, that lichen substance diminish the amount of chloroplastic manganese through a chelation progress.

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Address:

Khalid BOUAID & Carlos VICENTE
Laboratory of Plant Physiology, The lichen Team
Faculty of Biology, Complutense University
E-28040 Madrid
Spain
E-Mail: cvic@eumax.sim.ucm.es

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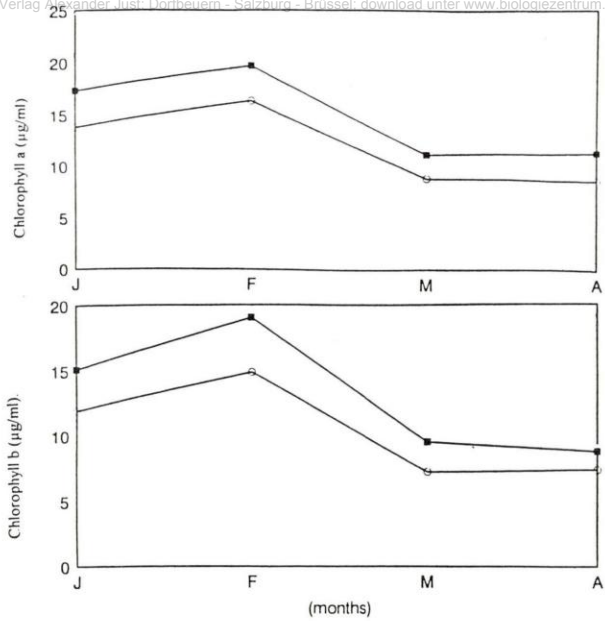


Fig. 1: Concentration of chlorophyll a and b in *Quercus rotundifolia* leaves without (■) and with (O) epiphytic lichens, measured from January to April.

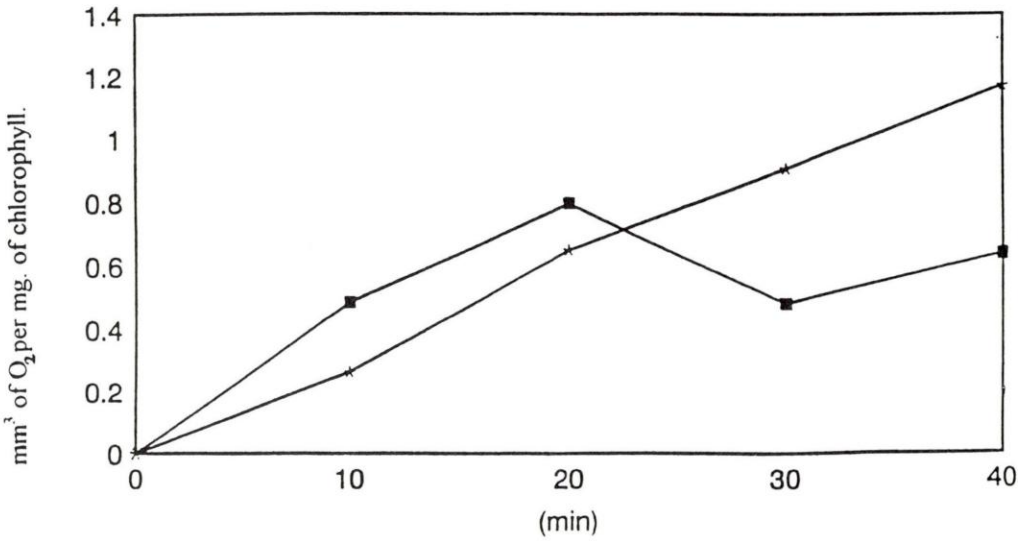


Fig. 2: Hill reaction activity of *Quercus rotundifolia* leaves as influenced by epiphytic lichens, Oxygen liberation through the Hill reaction from isolated chloroplasts of *Quercus rotundifolia* leaves without (*) and with (■) epiphytic lichens.

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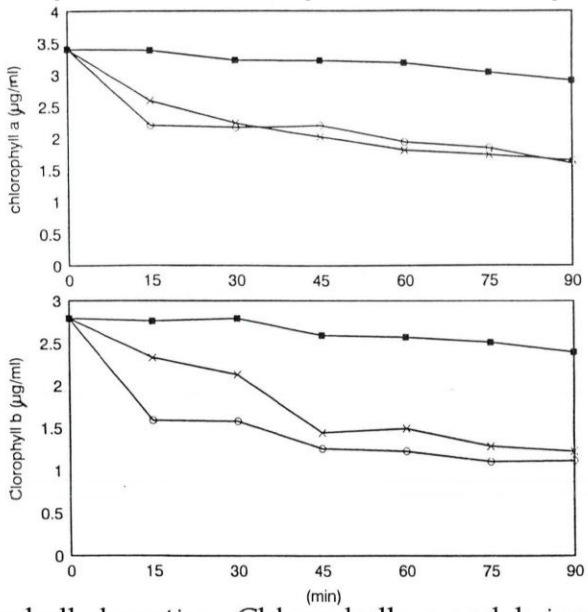


Fig. 3: Chlorophyll degration. Chlorophylls a and b incubated with 0.23 mg.ml^{-1} (X) and with 0.1 N HCl (O). (■) control without additives.

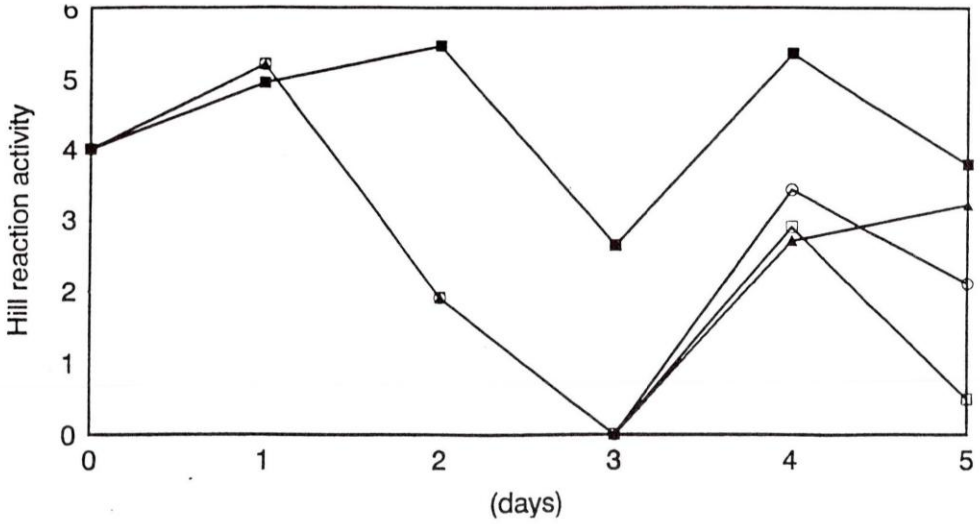


Fig. 4: Hill rection in chloroplasts of *Quercus rotundifolia* leaves isolated from:
(■) branches incubated for 5 days on 1 mM $NaHCO_3$.
(□) branches incubated for 5 days on 1 mM $NaHCO_3$ containing 14 µg. ml^{-1} usnic acid.
(O) branches incubated for 5 days on 1 mM $NaHCO_3$ containing 14 µg. ml^{-1} usnic acid and 0.2 mM $MgCl_2$ was added at the second day.
(▲) branches incubated for 5 days on 1 mM $NaHCO_3$ containing 14 µg. ml^{-1} usnic acid and 0.2 mM $MnCl_2$ was added at the second day.

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