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AIR POLLUTION DAMAGE IN *PARMOTREMA RETICULATUM* (LICHENES) THALLI, VIEWED WITH FLUORESCENCE MICROSCOPY

Riassunto — *Danni da inquinamento atmosferico ai talli di Parmotrema reticulatum* (Lichenes). *Studio in microscopia a fluorescenza.* — *Parmotrema reticulatum*, un lichene comune nella Liguria orientale, mostra alcuni sintomi di danneggiamento, clorosi e alterazioni della tessitura tallina, noti come effetti visibili dell'inquinamento atmosferico.

L'esame dei talli in microscopia a fluorescenza suggerisce una alterazione fisiologica dello stato simbiotico.

I talli danneggiati si distinguono facilmente dai sani per la comparsa di una anomala autofluorescenza gialla, a localizzazione prevalentemente corticale, la cui intensità aumenta parallelamente al peggioramento delle condizioni di vitalità.

Le analisi cromatografiche non mostrano variazioni significative del chimismo.

Abstract — *Parmotrema reticulatum*, a common species in eastern Liguria, is endangered by air pollution showing a wide variety of visible injury symptoms.

Fluorescence microscopy examination suggests physiological alterations of the symbiotic relationship. An acetone soluble material giving a yellow fluorescence appears mainly in the cortex. Its fluorescence intensity increases with increasing levels of air pollution as judged by the modification of the external aspect and the vitality of algal cells.

Chemical analyses, carried out by thin layer chromatography (TLC) show no significant variations in thallus chemistry.

Key words — Lichens, *Parmotrema reticulatum*, air pollution, fluorescence microscopy.

Introduction

Exposure to polluted air leads to visible injury symptoms in the thallus of pollution sensitive lichens (Hawksworth and Rose, 1976).

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Common symptoms are widespread discoloration (chlorosis) and alterations in thalline texture (lobe shriveling, contraction, decrease in thickness and substratum detachment). The chlorosis is caused by damage to algal cells. Its extention can be estimated either as the percentage of plasmolyzed cells (RAO and LEBLANC, 1966) or as the vital condition of the algae, observed on the basis of fluorescence of chlorophyll a (KAUPPI, 1980; KAUPPI and MIKKONEN, 1980) in thallus cross sections.

In the opinion of LEBLANC and RAO (1973), the modifications of the thalline texture are due to an upset moisture balance which can be provoked by a waxy material covering the outer cortices.

As referred by these authors (1973), this substance was insoluble in water and soluble in acetone and gave a shiny appearance to the upper surface of the thalli when observed in reflected light.

On the hilly eastern Liguria coast, *Parmotrema reticulatum*, characteristic of a lichen union which grows in humid evergreen mediterranean woods (NIMIS and SCHIAVON, 1986), is very common although it is rapidly disappearing from many areas in Liguria.

It is already absent in sites near large urban and industrial centres and it is endangered in almost all other areas along the eastern coast, as is visible from the widespread injury symptoms of the thalli.

The aim of this work is to verify if these injury symptoms in *Parmotrema reticulatum* thalli may be studied by fluorescence microscopy.

MATERIAL AND METHODS

About 100 pieces of thalli of *Parmotrema reticulatum* (Tayl.) Choisi with various visible injury symptoms were collected on olive trees or holm oak in eastern Liguria on the outskirts of Sestri Levante, a small tourist town on the Ligurian sea of about 20000 inhabitants. The sources of pollution are domestic heating plants and above all a small industrial centre which discharges about 700 tons of $SO2$ into the atmosphere each year.

In the area immediately surrounding Sestri Levante, as one goes further away from the town, a fairly rich lichen flora, with undamaged samples, can be observed.

In the chosen area a more precise record of $SO2$ emission and the delimitation of defined $SO2$ pollution zones, are lacking.

The fact that going further away from the town, the external aspect of the thalli improved and the damage symptoms became fewer until they disappeared, seemed indicative and sufficient for our study.

It was therefore possible that the situation reported by KAUPPI and MIKKONEN (1980) could present itself. According to these authors the external appearance of lichen thalli reflects the mean level of air pollution to the extent that is possible to trace the distribution of air polluting substances on this basis alone.

In order to obtain consistent samples the preparations were always made from the tip of the thallus lobe. Since their thickness is of little importance in epi-illumination fluorescence microscopy the sections were cut by hand from the fresh material to test the conditions of algal cells. At the same time small portions (1×1 cm) of thalli from the same samples were observed directly under the fluorescence microscope, using it as a stereomicroscope. In this way it was possible, with a low magnification, to observe the fluorescence of the upper surfaces of the thalli at various damage levels.

Microscopic examination was made with a Leitz Dialux 22EB microscope, equipped with a Ploem-Opak epi-fluorescence apparatus. The light source was a HBO 50 W Hg vapour lamp. H2 filter block (incident light 390-480 nm) was used throughout.

The samples were analyzed by thin layer chromatography (TLC) according to the standard method for lichen substances discussed by CULBERSON and AMMANN (1979).

RESULTS

The colour of *Parmotrema reticulatum* thalli progressively changed from greyish green to whitish grey as one moved from an area of low or no pollution, 4-5 Km away the town, to one of higher pollution level in the sites near the town. Also there was a gradual increase in the zone of discoloration in the thalli going from lower to higher levels of pollution. In no case did the upper surfaces have a shiny appearance if observed in reflected light.

Nearer the town the peripheral regions of lichen thalli were often shriveled and cracked.

In our experiments the sections of thalli collected in sites with a low pollution level, show that almost all the algae emit a strong red radiation. On the other hand the sections of thalli collected in areas closer to the source of pollution, show, parallel to the deterioration of the external aspect, a gradual increase in damaged algae. These become evident through the colour change of the fluorescence: from the dark red to orange and to an increasingly lighter yellow.

Furthermore our results demonstrate that in *Parmotrema reticulatum* not only the algal layer shows changes in fluorescence, but also the cortex and the medulla.

In healthy thallus cross-sections, where the algae appear fluorescent in vivid red, the remaining parts of the thallus are not fluorescent (Fig. 1).

Specimens collected in more polluted areas, show the appearance of a colourless substance giving a yellow fluorescence in all parts of the thallus (Fig. 3).

In specimens collected nearer the town, the algae are no longer visible when observed with fluorescence microscopy and the cortex seems to be the preferred area of accumulation of yellow fluorescent substance (Fig. 5).

Pre-treatment of the sections with acetone for a few minutes, completely removed the yellow fluorescent substance from all parts of the thallus.

When observed in fluorescence microscopy the upper surfaces of healthy specimens, collected in unpolluted sites, appear fluorescent in vivid red. This is due to the algae, visible under the transparent cortex (Fig. 2).

With a small magnification the surface appears grooved by a fine black network which separates the algae into clumps (Fig. 2). They are the pseudocyphellae which break up the continuity for the cortical layer.

In thalli collected in area closer to the town, together with changes of fluorescence in algal cells, the network of pseudocyphellae, in which a yellow fluorescent substance seems to be deposited, also becomes fluorescent (Fig. 4). From our observations it would appear that this deposit occurs very early and precedes all other damage symptoms. This aspect also seems to be the most commonly present in the samples of *P. reticulatum* collected, even in apparently healthy ones.

In the most highly damaged samples, the yellow fluorescent deposit in the pseudocyphellae becomes increasingly evident spreading throughout the thallus until it completely masks the underlying algae (Fig. 6).

The peripheral areas of the most damaged thalli appeared dark brown and no longer fluorescent when the upper surface was observed with a fluorescence microscope (Fig. 4).

The results of chemical assays in TLC through a comparison between the profiles of main diagnostic substances, no show significant variation in thallus chemistry in healthy and highly damaged samples of *P. reticulatum*.

DISCUSSION

Fluorescence microscopy tecnique, applied to specimens of *P. reticulatum* with various injury symptoms, confirms the observations of KAUPPI (1980) and KAUPPI and MIKKONEN (1980). Our experiments demonstrate that the vital conditions of the algae is a means suitable in forming a reliable impression of the state of pollution of a given area.

The most original aspect of our study concerns the appearance of a fluorescent deposit throughout the thallus which becomes heavier and more visible when the general conditions of deterioration increase.

This substance has the same solubility characteristic as the waxy material found by LEBLANC and RAO (1973) on the surface of *Parmelia sulcata* and *Physcia millegrana* thalli transplanted in various polluted zones. These authors found that the amount of waxy and other acetone soluble material in thalli consistently increased at increasing levels of pollution.

Our results show that together with the deterioration of the external condition of the thalli, chlorosis and texture, and the decrease in algal vitality, there is a progressive increase in the yellow fluorescence, mainly in the cortex. Contrary to results of LEBLANC and RAO (1973) we have not observed that the upper surface of the thalli is shiny in reflected light.

The abnormal yellow fluorescence appears very early in *P. reticulatum*, before all other external and internal symptoms and seems to be present from the beginning in connection with the pseudocyphellae, which represent cortical interruptions for the purpose of facilitating gaseous exchange between the inside of the thallus and the atmosphere.

Therefore the pseudocyphellae could be the main and/or most accessible means for the penetration of polluting substances into the lichen thallus.

Thus the fluorescent yellow substance could be interpretated as the first response to toxic substances.

Our chemical assays show that no alterations occur in the thallus chemistry, involving the main diagnostic substances. Spots of atranorin (cortical localization), salazinic and consalazanic acids (medullary localization) are visibile in TLC plates for every tested samples. This independently from the colour changes in fluorescence, from the vitality of algal cells and from the alteration in thalline texture.

On the other hand in TLC conventional conditions, the yellow fluorescent substance, observed by our microscopical examination, may be no more visible.

Other studies and a suitable standardized TLC method are needed to visualize the substance revealed by fluorescence microscopy.

At present it is difficult to relate the appearance of the observed fluorescent material to an alterated synthesis of lichen substances.

However these, although belonging to the secondary metabolism of the fungus, are controlled by the alga which characterizes their specificity (CULBERSON and AHMADJIAN, 1980). MOSBACH and SCHULTZ (1971) have observed that damage to the alga brings about modifications in the secondary metabolism of the fungus in *Lasallia pustulata*. This could cause the formation of phycotoxic substances in the thalli, alien to symbiosis (CULBERSON and AHMADJIAN, 1980).

Our observations show that a alteration in the lichen metabolism is visible by the appearance of a yellow fluorescent substance in damaged samples, absent in undamaged ones in which algal cells have a good vitality.

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BIBLIOGRAFIA

CULBERSON C.F., AHMADJIAN V. (1980) - Artificial reestablishment of lichens. II. Secondary products of resynthetized *Cladonia cristatella* and *Lecanora chrysoleuca*. *Micologia*, **72**: 90-109.

CULBERSON C.F., AMMANN K. (1979) - Standardmethode zur Dunnschichchromatographie von Flechtensubstanzen. *Herzogia*, **5**: 1-24.

HAWKSWORTH D.L., ROSE F. (1976) - Lichens as pollutions monitors. E. Arnold, London.

KAUPPI M. (1980) - Fluorescence microscopy and microfluorimetry for the examination of pollution damage in lichens. *Ann. Bot. Fenn., 17*: 163-173.

KAUPPI M., MIKKONEN A. (1980) - Floristic versus single species analysis in the use of epiphytic lichens as indicators of air pollution in a boreal forest region, Northern Finland. *Flora,* **169**: 255-281.

LEBLANC F., RAO D.N. (1973) - Effects of sulphur dioxide on lichen and moss transplant. *Ecology,* **54**: 612-617.

MOSBACH K., SCHULTZ J. (1971) - Studies on lichen enzymes. Purification and properties of orsellinate decarboxylase obtained from *Lasallia pustulata. Eur. J. Biochem.,* **22**: 485-488.

NIMIS P.L., SCHIAVON L. (1986) - The epiphytic lichen vegetation of the tyrrhenian coasts in central Italy. *Ann. Bot.* (Rome), **14**: 39-67.

RAO D.N., LEBLANC F. (1966) - Effects of sulphur dioxide on the lichen algae, with special reference to chlorophyll. *The Bryologist,* **69**: 69-75.

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TAVOLA I

Fluorescence changes in cross sections (Figs. 1, 3, 5) and in upper surfaces (Figs. 2, 4, 6) of *Parmotrema reticulatum* thalli, showing increasing levels of pollution damage.

Figs. 1, 2 - Aspects of the thallus in a healthy sample.

Fig. 1 - Algal cells showing a vivid red fluorescence, no other fluorescence is visible in the remaining parts of the thallus. × 320.

Fig. 2 - Red fluorescence of algal cells visible through the cortex. Note the black reticulum of pseudocyphellae. × 63.

Figs. 3, 4 - Aspects of the thallus in a damaged sample. Injury symptoms increase as one move from left (inner zone of the thallus) to right (outer zone) on the micrographs.

Fig. 3 - Fluorescence of algal cells turns from red to yellow-orange. An increasingly yellow fluorescence masking algal cells can be observed in the entire thallus. × 320.

Fig. 4 - A parallel change of fluorescence occurs as in the previous figure. The reticulum of pseudocyphellae becomes evident through a yellow deposit. × 63.

Figs. 5, 6 - Aspects of the thallus in a heavily damaged sample.

Fig. 5 - Algal cells fluoresce yellow and are only visible with difficulty. The yellow deposit occuring in the whole thallus appears to preferentially accumulate in the upper cortex. × 320.

Fig. 6 - The yellow fluorescence of algal cells appears masked by the yellow fluorescent deposit spreading out from a pseudocyphella. × 320.

TAVOLA I