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Chlorophyll fluorescence of epiphytic lichens under acid, ammonium and fungicide stress – a comparison of laboratory and field results

Kerstin Lübke, Horst Tremp

Abstract

Fungicide treatment (dithiocarbamate) caused a significant reduction of the quantum yield of photochemical energy conversion of the tested lichen species *Parmelia sulcata* and *Parmelia subrudecta* under laboratory conditions. Especially *P. subrudecta* showed a rapid decrease of photosynthetic capacity in between one week and was more susceptible to fungicide stress compared to *Parmelia sulcata.* Over the time span of four weeks, sulphuric acid and ammonium sulphate induced no significant changes of fluorescence parameters.

At natural stands (lichens on apple tree bark) the same fungicide was applied. Here no vitality-decline (visual verification, yield parameter) was detectable. So results from laboratory are not transferable on lichens living under natural habitat conditions, because removing lichens from tree bark causes additional stress to species. Results obtained from ecotoxicological laboratory experiments should be carefully proven and if possible completed and verified under more realistic background situations.

Key words: **lichens, chlorophyll fluorescence, quantum yield, *Parmelia sulcata*, *Parmelia subrudecta*, fungicide, ammonium, sulphuric acid**

Introduction

Lichens are suitable bioindicators of air pollution, because of their high sensitivity. In particular on acid loads they react very sensitive and far under immission limit values, which are designed predominantly for health protection purposes of humans (WIRTH 1987; KREEB et al. 1981, ZIERDT 1997). Already in the 19th century could be shown, that lichens react on air pollutions (GRINDON 1859; NYLANDER 1866) and can be used as bioindicators (ARNOLD 1891).

Lichens take up nutrients and pollutants over their entire surface directly from air or from precipitation. Pollutants might be enriched over long periods in the lichen thallus even in winter when metabolism of lichens keeps still active. Today environmental impacts in

Central Europe occur relatively rare at short high concentrations, but rather long-term in low concentrations. STEUBING & KIRSCHBAUM (1982) stated out, that lichens are damaged particularly by long-term, sometimes by very small immission effects, whereas higher plants suffering rather by short term high loads.

A clear quantitative relationship exists between chlorophyll fluorescence and the photosynthetic energy conversion (SCHREIBER 1997). Further it gives informations about light absorption, transfer of energy, and efficiency of electron transport. The pulse amplitude modulation technique (PAM) and the saturation pulse method permit laboratory and field application as well.

Lichens of genus *Parmelia* on barks of domestic tree species, often supply the base for immission-ecological investigations. In the German guideline for mapping lichen occurence (VDI 1995) also fruit trees are specified, because in the free landscape they often are the only carrier trees for lichens. The production of quality fruits requires intensified application of pesticides, especially fungicides. Therefore in the VDI recommendations it's pointed out that mapping of fruit trees from intensive cultivation is not admissible.

More exact notes, for example regarding the degree of intensity on which uninfluenced lichen vegetation still occur are missing, as same as which distances to intensive fruit gardens should be kept. Over the effect of pesticides on lichens as non-target organisms only few hints (BARTOK 1999) are given, although on lichens as symbiosis between fungi and algae a highly specific effect should be expected. This working hypothesis was checked by loading the lichens *Parmelia sulcata* and *Parmelia subrudecta* under laboratory as well as under field conditions. We applied Mancozeb (dithiocarbamate), a fungicide frequently used in orchards. By using the chlorophyll fluorescence technique the question was refined whether before occurring visible damage the effect of pollutants on lichens can be detected. The question was extended by applying classical air pollutants as sulphuric acid and ammonium of relevant ambient concentrations.

Materials and methods

As test organisms the two foliose epiphytic lichens *Parmelia sulcata* and *Parmelia subrudecta* were chosen. These species show a wide ecological valency. Further they frequently occur in the Central European region.

For measuring chlorophyll fluorescence of lichens the MINI PAM Fluorometer of Heinz Walz GmbH was used. It is optimally applicable for field investigations. If not specified the effective quantum yield ($\Delta F/Fm$) was measured, also indicated as Yield-parameter. The connection between Fluorometer and investigation object is made with a glass fiber optic, which serves as light conductor. A miniature fiber optic of 2 mm diameter was used. The distance between fiber tip and sample was approximately 4 mm in order to achieve a homogeneous recording area and a appropriate signal amplitude. The measurements in the laboratory were conducted under weak light conditions of $1-5\,\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperatures around 20°C.

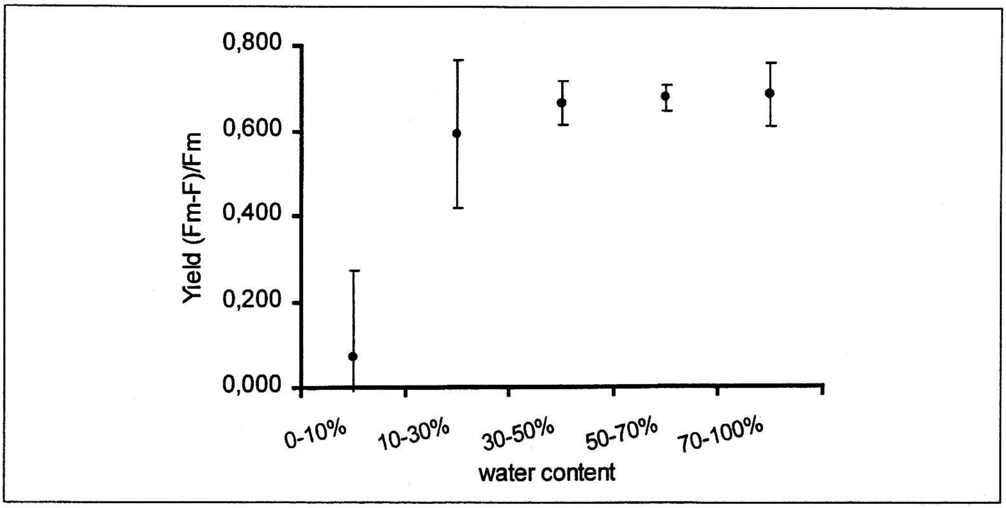

Fig. 1. Yield factor and standard deviations in dependence of classified thallus water content of *Parmelia sulcata* (n = 150).

The range of lichen water content in which the method provides reliable results had to be determined first. For that purpose the lichens were saturated on wet tissue paper in closed petri dishes. Desiccation of lichens occurred after opening petri dishes. In dependence of desiccation-time different water contents of lichens were obtained. After fluorescence measurement the fresh weight of lichens was determined then the lichens were dried (100 °C; 12 h) and weighted again. This preliminary experiment shows, that measurements of chlorophyll fluorescence can be conducted over a wide range of thallus water content between 20 – 100 % of maximum water content (Fig. 1). In this range measured Yield values varied between 0,65 and 0,71. Below 20 % Yield parameter decreased rapidly.

Parmelia sulcata and *Parmelia subrudecta* were taken from the bark of apple trees and cleaned. The lichens were divided into four groups of ten lichens. The size of the thallus was approximately 2 cm^2 with a mean weight of 0,12 g.

As test substances sulphuric acid of pH 3, ammonium sulphate solution (5000 $\mu mol \cdot l^{-1}$ NH_4^+) and the fungicide Dithane Ultra™ (Mancozeb 2 g/l; dithiocarbamate), frequently used in fruit-growing, were applied on lichens. The pollutants were applied before every days measurement in order to moisten and re-activate the lichens. In each case the applied quantity sprayed on thallus was approximately 0,14 ml. Yield measurements took place 15 minutes later. After this time lag the control group showed highest observable yield-values. In between pollution and measuring-cycle, the lichens were exposed under ambient conditions, protected against rain and direct sunlight. The experiment was conducted over three months. Altogether 26 measuring cycles were implemented.

The measurements in field were executed in extensive managed meadow orchards where no fertilizers and fungicides were applied. Single trees on which the two lichens grew on

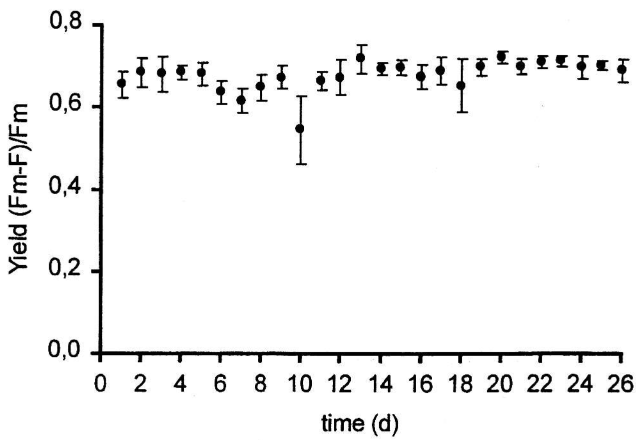

Fig. 2. Mean effective quantum yield and standard deviations (n = 10) of ammonium sulphate (5 mmol · l^{-1} NH_4^+) treated *Parmelia sulcata*.

their natural micro-sites were selected. On every tree three to four lichens were marked with coloured needles. Pollutants and number of replicates (n = 10) were the same as in the laboratory experiment. Over three months the yield-measurements took place two to three times a week. This was done before sunrise, in order to operate under weak light conditions. Apart from fluorescence parameters relative air humidity, air and leaf temperature and the photosynthetic active radiation (PAR) was measure at the same time.

Results

There was no observable effect on the base of the average effective quantum yield values of *Parmelia sulcata* and *Parmelia subrudecta* treated with sulphuric acid or ammonium sulphate solution over the time span of our laboratory experiment. The effective quantum yield kept in the range of 0,61 and 0,71. Within the standard deviations there are no larger fluctuations, only the values of *Parmelia subrudecta* treated with sulphuric acid showed a broader dispersion around the average value. The fluorescence parameters of the control groups (Yield 0,65–0,71) corresponded with these values, i.e. a severe influence of the pollutants sulphuric acid and ammonium sulphate was not observable (Fig. 2).

The lichens polluted with the fungicide Mancozeb showed a clear reduction of the effective quantum yield. The damage of the lichens could be determined both on the basis of the fluorescence data and in a late stage optically. Decreased vitality first could be observed on *Parmelia subrudecta* already after the eighth treatment (Fig. 3). Yield values of this species showed significant differences to the control group, whereas effects of *Parmelia sulcata* on fungicide treatment occurred later. Here significant differences could be found in the last eight measuring days (Fig. 4).

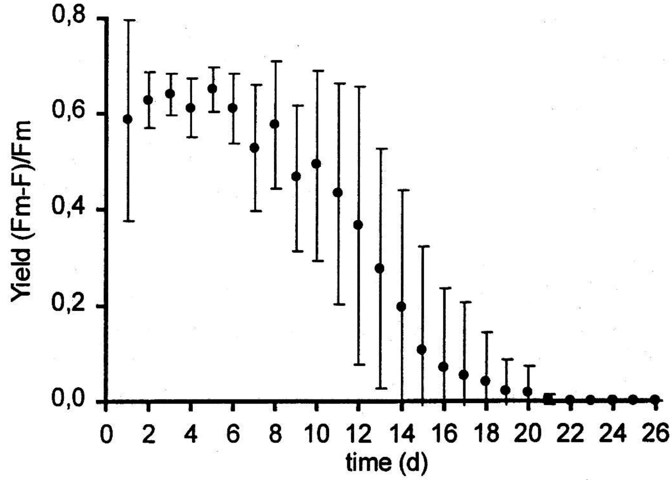

Fig. 3. Mean effective quantum yield (n = 10) and standard deviations of fungicide stressed *Parmelia subrudecta.*

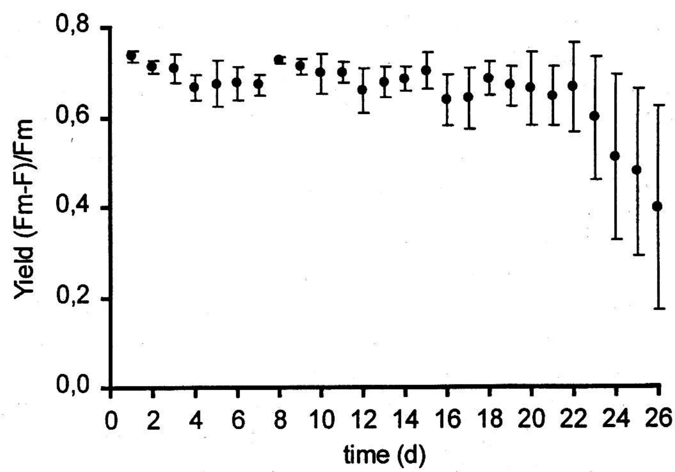

Fig. 4. Mean effective quantum yield (n = 10) and standard deviations of fungicide stressed *Parmelia sulcata*.

The lichens examined in the laboratory changed also in colour and structure (Tab. 1). The control group and ammonium sulphate treated lichens didn't show any modifications. Only slight bleaching of thallus was found in *Parmelia subrudecta*.

Tab. 1. Detectable visual damage on *Parmelia sulcata* and *Parmelia subrudecta* treated with fungicide under laboratory conditions.

Parmelia sulcata	***Parmelia subrudecta***
- red-brown spots - red-brown at the thallus margin and in the middle of thallus	- yellow-white spots - yellow-white at the thallus margin
colour: fading, then brownish-grey structure: thinner, softer	colour: fading, then yellowish-grey structure: getting extremely thin, decomposing

In field at any treatment no optical modifications of the lichens could be observed. Also chlorophyll fluorescence data showed no differences to the control group. Quantum yield values range between 0,6 to 0,7; the calculated standard deviations are large, significances between the groups of lichens treated with pollutants and the control group occur sporadic but irregularly, so that a well-defined pollutant effect on the parameter chlorophyll fluorescence on both lichen species could not identified.

Discussion

Most lichens possess the special ability to activate their photosynthesis after humidification rapidly (Nash 1996). Also Lange et al. (1989) come to the conclusion, that green algae lichens show a remarkable constancy of relative fluorescence and the yield value. Numerous investigations (Gauslaa 1996; Nash 1996) showed, that lichens are photosynthetically active already within shortest time after water application, thus chlorophyll fluorescence is assignable. After humidification dry lichen-thalli reached saturation of approximately 200 % (Lange et al. 1989) within a few minutes. Experiments of Gauslaa et al. (1996) indicated that modifications of the yield values are affected to a lesser extent by varying water contents, than by sample preservation.

The temporal shift and the observed fluctuations in the beginning of pollutant effects point to individual differences of the thalli and different toxicological tolerance of the species. Also Alstrup (1991) showed, that the sensitivity varies in dependence of applied pesticides and lichen species. Brown et al. (1995) determined, that no general statements or predictions can me made, because each chemical affects lichen species in a different way. Physiological processes like respiration and photosynthesis can be restrained, stimulated or uninfluenced (Brown et al. 1995). Alstrup (1991) stated out, that application of herbicides lead to far fewer damage than fungicides. But in general, agricultural activities always cause negative effects on the lichen flora of an area, nevertheless the reactions of the lichens to chemicals frequently used in agriculture cannot be generalised, since most studies supply only reduced predictions about the respective chemical or examined lichen species (Brown et al. 1995).

As we could show direct comparisons between the field and laboratory experiment are difficult. In short time experiments, the results from laboratory are hardly comparable and transferable on lichens living under natural habitat conditions. The lichens in the laboratory are more sensitive to the pollution treatment. The natural environmental factors seems to have influence on the resistance of the lichens. Additional, stress caused by removing from the bark, the measurement and the preservation can influence the results. Compared to normal application intervals of fungicides even in intensive managed orchards, which may reach 15 and more times over several months our short interval fungicide treatments under field conditions seems strengthened. Nevertheless the two investigated lichen species showed a not expected high tolerance against fungicide stress. Whereas the laboratory results indicated the more specific toxicity of a fungicide to lichens compared to classical pollutants like sulphur acid and ammonium.

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