Some Physiological Effects of Biocide Treatment on the Lichen *Pseudevernia furfuracea* (L.) Zopf.

NATALIJA VIDERGAR-GORJUP[1], HELENA SIRCELJ[2], HARDY PFANZ[3], and FRANC BATIC[2*]

[1]*Community of Zagorje ob Savi, Cesta 9. avgusta 5, 1410 Zagorje ob Savi, Slovenia;*
[2]*Agronomy Department, University of Ljubljana, Jamnikarjeva 101, 1001 Ljubljana, Slovenia, Tel. +386-1-4231161, Fax. +386-1-4231088, E-mail. franc.batic@bf.uni-lj.si;*
[3]*Institut für Angewandte Botanik, Universität Essen, Universitätsstrasse 5, 45117 Essen, Germany*

Abstract

Detrimental effects of biocides on epiphytic lichens are known from mapping studies but their effects at the physiological level are not well studied. *Pseudevernia furfuracea* (L.) Zopf. thalli were transplanted from a natural forest habitat (*Abieti-Fagetum dinaricum*, Tregubov, 1957) to experimental plots of the Agronomy Department of the University of Ljubljana to study the effects of biocide treatment on lichens in intensive apple cultivation. The response of this lichen to the fungicide Score 250EC was also studied under laboratory conditions and by spraying in the field. Net photosynthesis, chlorophyll fluorescence and pigment content (chlorophyll a and b, β-carotene, lutein and zeaxanthine) were analysed. Immersion of lichen into the fungicide solution had little or no damaging effect on the optimal quantum yield of photosystem II and on the concentrations of total chlorophyll, lutein, β-carotene and zeaxanthine, but showed a significant depression of net photo-

*The author to whom correspondence should be sent.

synthesis together with a decrease of effective quantum yield of photosystem II. Spraying with the same fungicide during the vegetation period significantly decreased net photosynthesis in July and August but had a very slight effect on optimal and effective quantum yield of photosystem II and on the content of pigments except zeaxanthine, the concentration of which increased after sprayings. The response of *Pseudevernia furfuracea* to a mixture of biocides during the regular spraying programme in an intensive apple cultivation showed a decline in net photosynthesis from June to August and a small effect on the optimal and effective quantum yield of photosystem II. This treatment also resulted in a decrease of total chlorophyll from May till beginning of July but had a diverse effect on the content of lutein, β-carotene and zeaxanthine. The response of the lichen to the treatments in the field and laboratory conditions was partly different in type and extent of measured parameters due to the state of the lichen thalli under experiment, dose of biocide applied and interference of heterogeneity of lichen thalli and environmental conditions.

Keywords: *Pseudevernia furfuracea*, biocide treatment, net photosynthesis, chlorophyll
                    fluorescence, photosynthetic pigments, intensive apple cultivation, Slovenia

1. Introduction

Epiphytic lichens are generally considered to be good indicators of air quality. There have been many studies on the effects of SO_2, heavy metals, fluorides, nitrogen oxides, ozone and other industrial products on lichens (Richardson, 1988; Silberstein et al., 1996), but there is very little information on the effect of agrochemicals on lichens (e.g. Loppi and DeDominicis, 1996; Vagts et al., 1994; Benfield, 1994), and there have been even less studies on the physiological effects of agrochemicals on lichens and most of these have concentrated on a limited range of substances (DaSilva et al., 1975; Hällbom and Bergman, 1979; Brown et al., 1995; Shaaltiel et al., 1988; Alstrup, 1992; Brown, 1992; Modenesi, 1993; Jensen et al., 1999). Biocides are important in improving the productivity of modern farming but they may also have an impact on non-target organisms. The effects of agrochemicals upon lichens on fruit trees was also the subject of two studies in Switzerland (Ruoss, 1999). A mapping study in St. Gallen showed no significant influence of agrochemicals on the lichen flora on pear and apple trees, but in a second study the observations of lichens on untreated apple trees and on trees regularly treated with insecticide showed better vitality of lichens and higher species number on untreated trees. Alstrup (1992) reported an investigation of herbicide and fungicide damage to lichens growing on siliceous walls in Denmark. He showed that lichen sensitivity to biocides differed between species and chemicals;

herbicides were often less damaging than fungicides. Bartók (1999) confirmed that lichen species showed a differential sensitivity to the nature and frequency of biocide applications and concluded that biocides were generally unfavourable for the growth of epiphytic lichens. Jensen et al. (1999) found no particular sensitivity of green algal lichen photobionts to photosystem II herbicides as compared to other algae, higher plant chloroplasts or protoplasts. In nature, they observed recovery from (damaging) treatment with Diuron within weeks. Brown et al. (1995) reported a slight temporary inhibition of respiration and photosynthesis of *Peltigera* caused by the fungicide Propamocarb. There was no evidence of membrane damage, no sign of recovery during the subsequent 24-h period and it is unclear why it should cause even a temporary depression of photosynthesis. In accordance with most available articles, we agree with Ruoss (1999) that even now the effects of agrochemicals on lichen physiology are poorly understood. It is doubtful that any generalizations can yet be made about lichen responses to agricultural chemicals because in most studies involved only a limited number of chemicals and lichen species were investigated. There will be considerable heterogeneity in responses when further studies are conducted (Brown et al., 1995).

In this paper we present some effects of a differential biocide treatment on the epiphytic lichen *Pseudevernia furfuracea* on the basis of measurements of net photosynthesis, chlorophyll fluorescence and pigment analyses. *Pseudevernia furfuracea* (L.) Zopf. is a widely distributed and relatively pollution-tolerant epiphytic lichen, which has recently been used in some ecophysiological studies (Piervittori et al., 1997; VanHerk, 1999; Kranner and Grill, 1994) and turned out to be sensitive to biocides (Ruoss, 1999). The species usually occurs on orchard trees in the extensive high stem type of fruit production but is absent from modern plantations in which small-stem fruit trees are planted in high densities The main aim of our study was to clarify the physiological responses of the lichen to a biocide treatment in an intensive apple orchard parallel by experiments using Score 250EC fungicide in the field and in the laboratory. Score 250EC is a systemic fungicide for suppression of fruit diseases caused by fungi such as *Venturia inaequalis*, *V. pyrlina* and *Podosphaera leucotricha*. The active substance is Diphenkonazol (250 g/l). The primary biochemical sites are fungal cell walls, where it suppresses the biosynthesis of sterols. It is not clear whether Score is toxic to algal cells. As concentrations needed to achieve a measurable depression of photosynthesis are two-to-three orders of magnitude higher than the maximal concentrations found in the environment (Jensen et al., 1999), we decided to perform the experiment with three times the manufacturers' recommended dosage, and lichens were sprayed with 0.045% solution of Score 250EC.

2. Material and Methods

Study area

The investigation was carried out at the experimental field of the Agronomy Department, Biotechnical Faculty, University of Ljubljana in Ljubljana. For the field treatments lichens were exposed and treated outdoors as presented later in the experimental design. Detached branches with adherent lichens were fixed on apple trees within the plantation and additionally to the bushes of hasel nut in the vicinity. Meteorological parameters and concentrations of air pollutants were therefore nearly the same at all exposure plots.

Lichen transplants

P. furfuracea thalli were collected on 1st April 1999 on mount Sneznik (northern part of the Dinaric mountains, Slovenia) at a location far from potential pollution sources. The branches with lichen thalli were collected from well-lit crowns of *Abies alba* and were then immediatlly transported to Ljubljana in plastic bags where they were randomly distributed into three groups. The thalli for the fungicide treatment were collected on 23th of April, 1999.

Experimental design and treatments

The study was carried out in the 1999 vegetation period, considering apple production. The influence of agrochemicals was investigated in an orchard experiment (1st group, orchard), where different types of biocides were used in apple production. Twigs with lichens were fastened to apple trees in light conditions similar to those found at the original site, and the lichens were sprayed with biocides during normal agricultural activities (Table 1). In this experiment apple trees and lichens were sprayed also with Folimat (0.1% solution, on 18th of June), Folifertil (0.2% solution; on 9th of June, 18th of June, 27th of June and 27th of July) and elemental sulphur (0.3% solution, on 18th and 27th of June) as normal treatments in modern apple growing. A second group of lichens was exposed on nearby hazel-bushes (*Corylus avellana*) and one week before each harvest was sprayed with a 0.045% water solution of Score 250EC fungicide (2nd, "sprayed" group). The third group of lichens (3rd, "control" group) was also exposed on hazel bushes in the vicinity but was not sprayed. Each month six samples of thalli from all three lichen groups were sampled randomly and net photosynthesis and pigment content were determined. An additional experiment with the same lichen species was carried out at the end of April when the outdoor experiment started. Lichens were first soaked in tap

Table 1.   Biocides used in the orchard in 1999

Pesticide	Type	Concentration (%)	Date of the treatment
Folidol	I	0.5	7.4.
Cuprablau	F	0.5	7.4.
Chorus 75WG	F	0.03	15.4.
Score 250EC	F	0.02	23.4., 11.5.
Dithane M45	F	0.2	23.4., 18.6.
Clarinet	F	0.1	1.5.
Captan	F	0.2	11.5., 9.6., 27.6., 27.7., 27.8.
Confidor	I	0.03	18.5., 12.7.
Mythos	F	0.1	18.5.
Delan	F	0.1	28.5., 12.7.
Karathane	F	0.1	28.5.
Basudin	I	0.15	9.6.
Systhane 6 FLO	F	0.07	9.6.
Nissorun	A	0.05	27.6.
Insegar	I	0.04	12.7.
Thiodane	I	0.1	27.7.
Mitac	I	0.3	27.7.
Zolone	I	0.25	27.7.
Euparen	F	0.2	12.8.

F = fungicide, I = insecticide, A = acaricide.

water for 30 minutes and than divided into 2 groups which were immersed in a solution of the fungicide Score 250EC for 5 and 60 minutes. After that the lichens were removed from the solution, blotted with filter paper and immediately measured and analysed. The pH of the fungicide solution was 6.96 and did not change during the procedure.

Measurements of net photosynthesis

Net photosynthetic rates (Pn) of thalli can be a function of the ratio of young and old thallus material used (Kershaw, 1985). Therefore we tried to measure net photosynthesis on thalli with approximately the same ratio of young to old material. We expected a marked variability in gas exchange rates due to the different age of thalli, chlorophyll contents of the thalli and the differential development of the propagation phases, which heavily affected both CO_2 exchange and water relations (Tretiach and Carpanelli, 1992). Remnants of the substrate were removed from the thalli prior to measurements and the samples were submerged in distilled water for 30 minutes. After complete saturation

they were exposed to a photon flux density of (white light) 100 μmol m^{-2}s^{-1} for 30 minutes in order to allow the completion of the main part of resaturation respiration (Smith and Molesworth, 1973; Farrar and Smith, 1976). Excess water was removed by blotting the thalli with tissue paper and the samples were then placed in a cuvette illuminated with 500 μmol m^{-2}s^{-1} PAR. CO_2 exchange was measured using an infrared gas analyser in an open-flow ADC LCA-3 system (ADC, Hoddesdon, UK). Water loss from the illuminated lichen samples during the experiment was unavoidable. The CO_2 concentration ranged between 330 and 350 ppm. The CO_2 gas exchange rates were monitored continuously until maximal photosynthesis was reached. Only CO_2 gas exchange rates at optimal water content, i.e. when the CO_2 gas exchange was maximal, were used for data evaluation (see also Seel et al., 1992). Net photosynthesis was calculated on a dry weight basis (46 h, 60°C) and expressed in μmol CO_2 g^{-1}h^{-1}. Six replicates were made for each treatment.

Pigment analyses

Thalli were thoroughly cleaned from adherent parts of moss and bark fragments, frozen with liquid nitrogen and stored at –25°C until use. Later the samples were freeze-dried, homogenised to a fine powder in a ball mill and again kept frozen at –25°C until analysis. Pigment extraction was performed in dim light and at low temperatures. Degradation of chlorophylls (see also Brown and Hooker, 1977) was prevented by the addition of 100 mg $MgCO_3$ per 200 mg of lyophilised powder prior to the extraction with acetone. Additionally 200 mg polyvinylpolypyrrolidone per 200 mg of lyophilised sample was added. A Spectra Physic HPLC system was used for the analysis of photosynthetic pigments using the method developed by Pfeifhofer (1989).

Fluorescence measurements

From each treatment one twig with 20 (marked) lichen thalli was reserved for the fluorescence measurements. Prior to measurements, the twigs were carefully removed from the exposed trees and the lichens wetted with tap water for 30 minutes. Hydrated thalli were left at 20°C for 30 minutes in darkness before optimal quantum yield of photosystem II (Fv/Fm = optimal quantum yield of photosystem II) was measured with an Opti Science 500 Modulated Fluorometer on the marked thalli lobe. Effective quantum yield of photosystem II (Fv'/Fm' = effective quantum yield of photosystem II) was measured afterwards on the same lobes of the hydrated and well lit (300 μmol m^{-2}s^{-1} PAR) thalli. After the measurements the twigs with the lichens were further exposed at their previous sites for the following measurements.

Table 2.   Results of measurements of different parameters as measured after immersion of *Pseudevernia furfuracea* in a 0.045% solution of Score 250EC for 5 and 60 min (mean±SD; n=4)

Time (min)	Net ph. (μmol CO_2 $g^{-1}h^{-1}$)	Fv/Fm	Fv'/Fm'	Chl a (mg/g)	Chl b (mg/g)	Lutein (mg/g)	Zeaxanthine (mg/g)	β-Carotene (mg/g)
0	14.97 ±8.78	0.724 ±0.009	0.413 ±0.056*	0.688 ±0.072	0.415 ±0.050	0.264 ±0.033	0.062 ±0.024	0.061 ±0.007
5	Negative	0.710 ±0.042	0.295 ±0.057	0.627 ±0.040	0.366 ±0.040	0.235 ±0.026	0.034 ±0.004	0.058 ±0.023
60	Negative	0.695 ±0.096	0.254 ±0.042*	0.667 ±0.116	0.382 ±0.035	0.239 ±0.027	0.043 ±0.009	0.074 ±0.018

Fv/Fm = Optimal quantum yield of photosystem II; Fv'/Fm' = Effective quantum yield of photosystem II. * = Significant differences at $p<0.05$.

Statistical   evaluation

The data obtained were statistically processed with Statistica (Windows 5.0 package).   The results were evaluated by analysis of variance (ANOVA) and Turkey's HSD test for unequal n (Spjotvoll/Stoline test) for determining significant differences   at $p<0.05$.

3. Results

The data presented comprise measurements of chlorophyll fluorescence, net photosynthesis and pigment analyses (Table 2).

After immersion of the thalli in the solution of Score 250EC the decrease of the ratio Fv'/Fm' and the depression of net photosynthesis were very severe. Furthermore a decrease of ratio Fv/Fm was observed, and the content of several photosynthetic pigments decreased (Chl a, Chl b, lutein and zeaxanthine) but the results were not statistically significant.

The values of Fv/Fm (Fig. 1) did not change much during the treatment. From April to October the maximal photochemical efficiency of PSII increased slightly in all treatments, but the increase was significant only for the control thalli.   Fv/Fm was lowest in samples from the orchard, where ANOVA
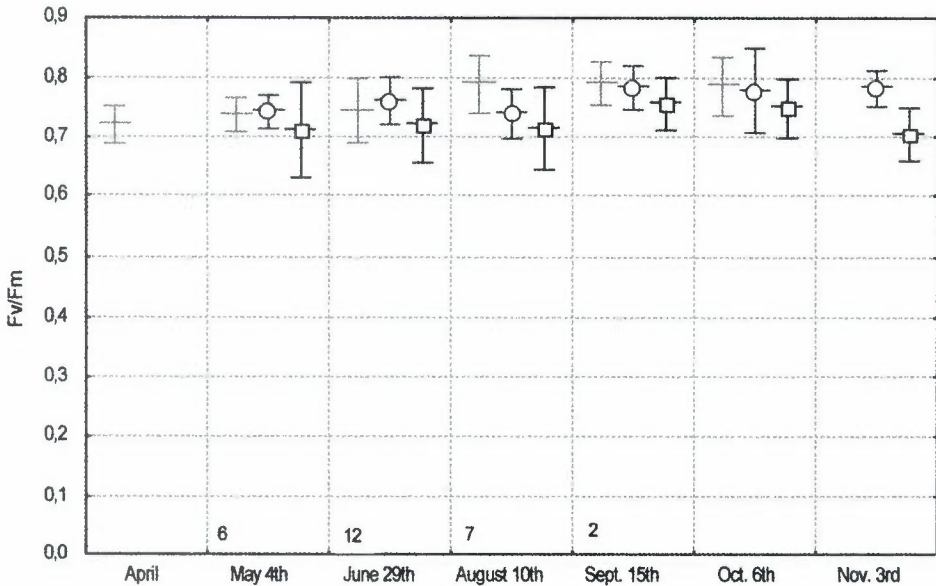
Figure 1.   Seasonal measurement of Fv/Fm (mean±SD; n=20) of *Pseudevernia furfuracea*. Control lichens (+), sprayed lichens (circle), lichens exposed in the orchard (square). Fv/Fm = optimal quantum yield of photosystem II. The numbers above the abscissa indicate the number of biocides applied in the orchard in the period between two successive lichen sampling.

showed that the differences between untreated control thalli and thalli in the orchard treatment were significant in August and September.

The pattern of the effective quantum yield of photosystem II (Fig. 2) was comparable to the values of Fv/Fm (Fig. 1), but the actual values were lower. Only in April were extremely low values of Fv'/Fm' recorded. A significant decrease in Fv'/Fm' was evident at the 5% significance level in May in the orchard treatment and in August in the control samples. A statistically significant increase in the effective quantum yield of photosystem II was detected in all three treatments in September.

During the whole treatment, net photosynthesis of the treated thalli (control, sprayed, orchard) remained more or less constant. Significantly higher net photosynthesis in control samples was measured only in August and significantly lower in October. This can be explained partly by the heterogeneity of lichen thalli and even more by the different activity of lichens during the summer months due to water availability and the time when the measurements were carried out. Comparison of net photosynthesis among
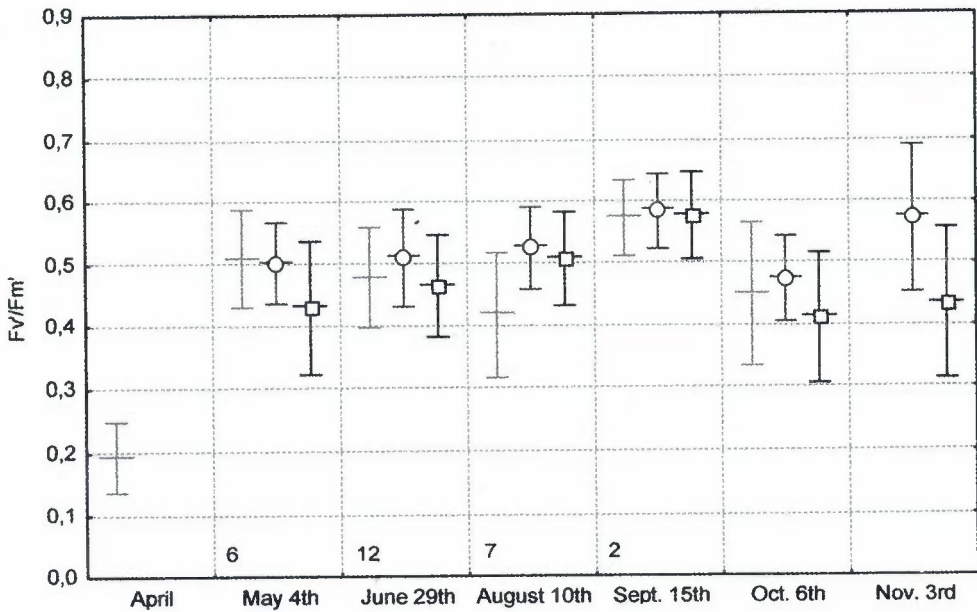
Figure 2. Seasonal changes of the ratio Fv'/Fm' (mean±SD; n=20) of *Pseudevernia furfuracea*. Control lichens (+), sprayed lichens (circle), lichens exposed in the orchard (square). Fv'/Fm' = effective quantum yield of photosystem II. The numbers above the abscissa indicate the number of biocides applied in the orchard in the period between two successive lichen sampling.

Table 3. Ratio of chlorophyll a/b of thalli of *Pseudevernia furfuracea* exposed under three different treatments (n = 6)

	Control (mean±SD)	Sprayed (mean±SD)	Orchard (mean±SD)
April 2nd	1.492±0.352	–	–
May 4th	1.463±0.202	1.253±0.187	1.539±0.181
June 1st	1.642±0.283	1.997±0.417*	1.210±0.169*
July 1st	1.445±0.305	1.370±0.255	1.369±0.158
July 27th	1.421±0.386	1.223±0.187	1.417±0.225
August 24th	1.573±0.560	–	1.639±0.293

* = Significant differences at p<0.05.
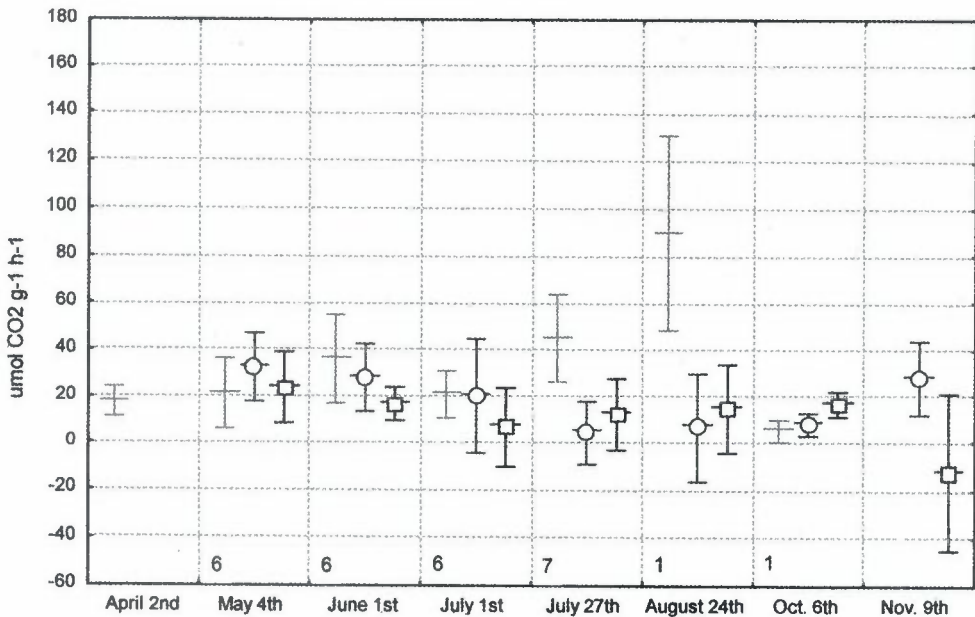
Figure 3.   Net assimilation rates (mean±SD; n=6) of *Pseudevernia furfuracea* measured
under standard conditions (T = 25°C, PAR = 500 μmol m^{-2}s^{-1}, CO_2 = 330–350
ppm. Control lichens (+), sprayed lichens (circle), lichens exposed in the orchard
(square). Above the abscissa of the graph are given the number of biocides
applied in the orchard in the period between two successive lichen sampling.

differently treated lichens at each sampling showed significant differences
between the control and orchard treatment in late summer and autumn. The
values measured on sprayed lichens were significantly lower in comparison to
the control in July and August (Fig. 3). These data are consistent with
fluorescence measurements where only the effect of combined biocide spraying
caused a statistically significant decrease of Fv/Fm.

Total chlorophyll content (Fig. 4) and the ratio of Chl a/b (Table 3) in
exposed thalli in the orchard significantly decreased in June. However, in this
month the ratio Chl a/b in lichens sprayed with fungicide also significantly
increased. This might be due to the high sensitivity of Chl b to oxidative stress
(Young and Britton, 1990). Total chlorophyll content in the sprayed lichens was
not significantly changed (Fig. 4). Considering chlorophyll content in control
thalli, there was a tendency to increase during the summer, but the variation
among the thalli was high. Spraying with different biocides in the orchard
had the biggest effect on chlorophyll content, while single fungicide treatment
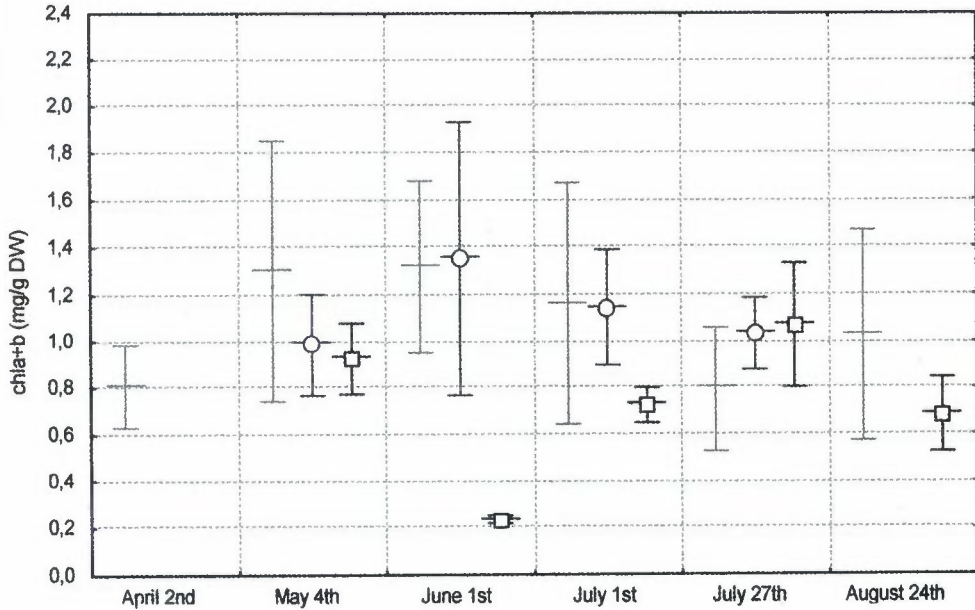decreased chlorophyll to a smaller extent. Very similar results were also

Figure 4. Total chlorophyll content (mean±SD; n=6) in *Pseudevernia furfuracea*. The experiment was carried out as described in Fig 1. Control lichens (+), sprayed lichens (circle), lichens exposed in the orchard (square).

obtained from analyses of luteine and β-carotene content. Samples treated with Score 250EC, showed a significant increase in the content of lutein and β-carotene during the treatment. In the orchard treatment the content of lutein and β-carotene remained almost always lower than in the control and sprayed samples, decreasing significantly in June and slowly rising towards the end of July.

The content of zeaxanthine (Fig. 5) was high in spring in the control before the beginning of the experiment. During the treatment, the control had always lower values in comparison to the treated lichens. In June the zeaxanthine content in sprayed lichens remained high, while values measured in the control and orchard thalli decreased significantly. In July the zeaxanthine content in the control thalli was low and contents in treated thalli were quite high, but the differences were not statistically significant. Generally higher values in treated lichens indicate that biocides acted as stress substances leading to an increase of zeaxanthine in order to prevent further destruction of the already low content of chlorophyll.
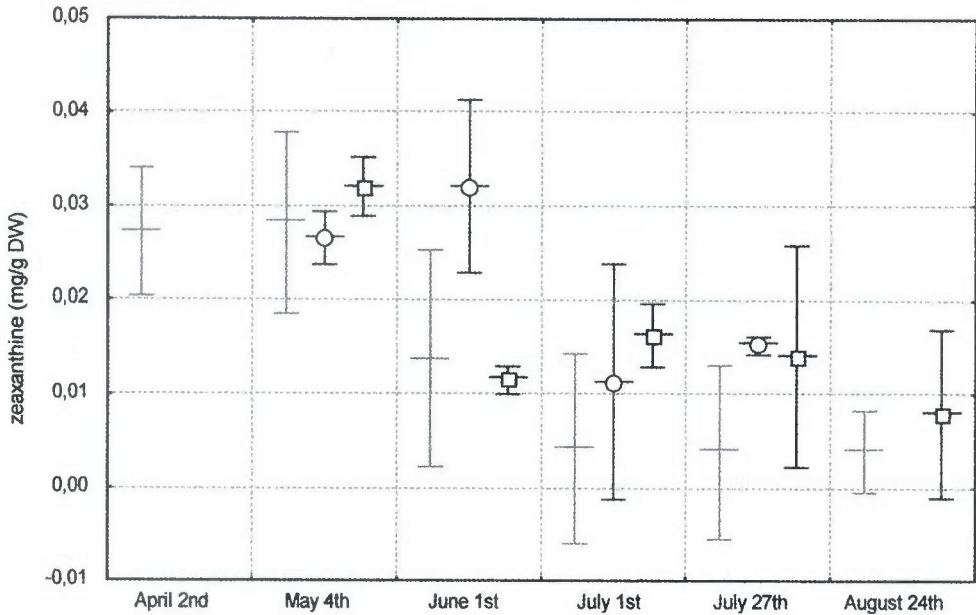
Figure 5. Zeaxanthine content (mean±SD; n=6) in *Pseudevernia furfuracea*. The experiment
was carried out as described in Fig 1. Control lichens (+), sprayed lichens
(circle), lichens exposed in the orchard (square).

4. Discussion

Environmental stress is often manifested by a decline in the Fv/Fm
fluorescence ratio (Lichtenthaler, 1988; Gauslaa et al., 1996). The ratio
variable to maximal fluorescence (Fv/Fm) reflects the maximal photochemical
yield of PS II centres and it correlates with the number of functional PSII
reaction centres (Catalayud et al., 1996). It is an indicator of the efficiency of
photochemical energy conversion in PSII (Sonesson et al., 1995; Demmig-Adams
and Adams, 1996). This parameter varies between 0 and 0.84; high values are
achieved in healthy systems, low values indicate damage or inactive states
(Jensen, 1994; Bilger et al., 1995). In the laboratory experiment, the control
thalli had an Fv/Fm ratio of 0.724 ± 0.009 (Table 2). Thalli immersed in a
solution of Score 250EC showed no significant decrease in the ratio Fv/Fm
indicating that Score 250EC did not directly impair the function of PSII. This
was also confirmed by the results of the thalli sprayed with Score 250EC in the
experimental field, where no statistically significant decrease of Fv/Fm was
measured (Fig. 1). In the orchard treatment Fv/Fm ratio was the lowest,

indicating a decrease in the photochemical efficiency of PS II and a lower vitality of the thalli due to the biocide treatment.

The actual efficiency of energy conversion in PS II is given by the expression Fv'/Fm' (effective quantum yield of PSII) and is regarded as the number of electrons transported per photon absorbed by PSII (Jensen, 1994; Bilger et al., 1995). Maximal values close to 0.82 can be obtained, which indicate high photosynthetic efficiencies (Seaton and Walker, 1990). The mean values of Fv'/Fm' in our experiment were between 0.2 and 0.6. In the thalli immersed in a solution of Score 250EC ANOVA showed a statistical difference in comparison to the control (Table 2), but in the field experiment, with the exception of August, when the control ratio was unusually low, no significant changes between control and sprayed lichens were found (Fig. 2).

A statistically significant decrease of net photosynthesis was observed in the thalli immersed in a solution of Score 250EC (Table 2) and in thalli sprayed with the same fungicide in July and in August (Fig. 3). From this we concluded that the negative impact of Score 250EC on photosynthesis of *P. furfuracea* showed up quickly, that its effect was quite long lasting, but, considering the results of pigment analyses, not very damaging. We do not know whether the depression was the result of direct impact on the alga or impact through a change in fungus metabolism. Modifications in fungus metabolism could cause the formation of phytotoxic substances in the lichen thallus (Culberson and Ahmadjian, 1980). In any case the decreased photosynthesis may be a symptom of cellular damage (Brown et al., 1995) of one or both symbiotic partners. Alstrup's (1992) microscopical investigations showed that both symbiotic partners were apparently affected at the same time. Biocide treatment of lichens in the orchard also lead to a considerable decrease in photosynthesis, which was measurable already in the early beginning of June and statistically confirmed at the end of July and in August.

Chlorosis and colour changes of treated *P. furfuracea* thalli (see also the work of Modenesi (1993), for *Parmotrema reticulatum* treated with Paraquat) were noticed in July, in August and in October. Damaged thalli normally turned decolorized or brown, and in some cases they peeled off the twigs (see also Alstrup, 1992). On some parts of the treated thalli black areas also occurred. The chlorosis can be easily explained as a destruction of chlorophyll mediated by biocides. The changes in colour could be linked to the presence of lichen substances (Hawksworth and Rose, 1976) and to secondary lichen metabolism. Although lichen acids belong to the fungal metabolism, their synthesis could be controlled by the alga; damage to the alga brings about modifications to the metabolism of the fungus, and vice versa (Culberson and Ahmadjian, 1980). The lowest concentrations of total chlorophyll, β-carotene and lutein were found in the lichens exposed in the orchard in the beginning of June and July (Figs. 4 and 5). The results are in accordance with the suggestion of photo-bleaching of

pigments, which is generally in the following order: β-carotene > lutein > chl b > chl a (Young and Britton, 1990). Our results confirmed the suggestion of Gauslaa et al. (1996) that Fv/Fm should probably be a more sensitive measure of early stress, due to unfavourable environmental conditions, than chlorophyll degradation. In the orchard a decrease in the ratio Fv'/Fm' was observed already in May, while the total chlorophyll content significantly decreased in June and apparently represents one of the later stages in photosynthetic damage.

Together with changes in chlorophyll fluorescence, the effect of biocide treatment in the orchard first became evident in the increased level of zeaxanthine in May. The spraying of lichens with Score 250EC appeared to be reflected in the statistically significant high concentration of zeaxanthine in June. In comparison to the control, the content of zeaxanthine in sprayed lichens was also high in July, but not significantly. With the exception of a relative high zeaxanthine content and a high chl a/b ratio measured in sprayed lichens in June, there were no other significant differences in the levels of pigments due to the treatment with Score 250EC. Many environmental stresses can limit the ability of plants to use light energy and induce increases in the levels of the xanthophyll cycle dependent energy dissipation. The accumulation of zeaxanthine is a way of dissipating excess energy non-radiatively, thus protecting the photosynthetic apparatus and especially the chlorophylls. In many environmental-stressors, particularly those affecting photosynthesis, the presence of zeaxanthine can be used as an indicator of stressed tissue (Young and Britton, 1990). After stress cessation consequent epoxidation of zeaxanthine to violaxanthine can take place (Demmig et al., 1988; Demmig-Adams and Adams, 1994). β-Carotene is the biosynthetic precursor of zeaxanthine (Adams and Demmig-Adams, 1992), so low concentrations of β-carotene, which often occurred in our samples with a relatively high zeaxanthine content, can be explained. All additional environmental stresses that lower the plant's photosynthetic rate increase the degree to which absorbed light can be excessive, increasing the need for energy dissipation (Demmig-Adams and Adams, 1996). In lichens the determination of zeaxanthine content also makes it possible to estimate the extent to which lichens have been exposed to potentially damaging illumination (Jensen et al., 1993). Exposure to a combination of stresses in the field can lead to the maintenance of high levels of zeaxanthine, which were also detected in the thalli of *P. furfuracea* exposed to the various biocides (Fig. 5) used in intensive apple cultivation (Table 1), and separately exposed to the fungicide Score 250EC.

The induction of these energy dissipative mechanisms can be reflected in a reversible down regulation of photosystem II activity, which can be measured as a decrease in the ratio of variable to maximum chlorophyll fluorescence, often correlated with formation of zeaxanthine (Thiele et al., 1998). Due to the

experimental design, we were not able to detect whether the increase in zeaxanthine content was related to an increase in the rate of radiationless energy dissipation as indicated by the decline in fluorescence, as suggested in Demmig et al. (1988) for higher plants.

5. Conclusions

Thalli of *Pseudevernia furfuracea* treated with Score 250EC showed significant changes in net photosynthesis and zeaxanthine content. In the field experiment the differences were first observed in June, with the highest effects found in early summer. Score 250EC appears to have only a limited damaging effects on chlorophyll fluorescence, on total chlorophyll content and on the concentrations of lutein and β-carotene.

An integrated biocide treatment in intensive apple cultivation partially resulted in a reduction of the efficiency of photochemical energy conversion in PSII. The decrease of net assimilation rates and total chlorophyll concentrations were other indicators of detrimental biocide effects, but not throughout the experiment. It seemed that environmental parameters strongly modified lichen response to biocide treatment considering these parameters. The responses of photosynthetic pigments, decreases in lutein and β-carotene content together with an increase of zeaxanthine content due to biocide treatment were also confirmed. Brown (1992) suggested that herbicides, used at normal concentrations and in volumes comparable to those of spray drift, usually have very limited inhibitory effects on lichens. It is difficult to make comparisons between previous results of biocide effects on lichens and ours since neither the agrochemicals, the time period and procedure of exposure, nor the lichen species were the same. There are many possible ways in which biocides may cause damage to lichens. Some changes are clearly temporary and may not cause permanent damage to the lichen, while others could cause permanent damage too, or result in death of the lichen (Brown et al., 1995). The great differences of lichen response to treatment with single fungicide under laboratory conditions and treatment by the same fungicide in the field and by the complex mixture of biocides in apple orchard in our experiment could be explained by the procedure of the experiment, dose of the biocide applied and environmental effects. In the laboratory, lichens were soaked by the solution with high fungicide concentration. In the field thalli were just sprayed by the fungicide and biocide mixture, the concentration of which was probably not high enough to cause such an effect like in the laboratory. Due to the heterogeneity of lichen thalli (age of thalli, genetic variation) and changing environment before, during and after biocide application the very different response of the lichen in the field could be understood. This is also the evidence

that great care must be paid to experiment design and to interpretation of results obtained under different conditions. Although we did not get the same results in lichen response in the field and laboratory conditions our results confirmed that intensive cultivation in orchards with direct application of biocides caused some physiological damage to *P. furfuracea* and that the chosen indicators of decreased vitality could explain to a certain extent the die-back of lichens in sprayed orchards, especially considering repeating application of biocides year by year not to mention the effects of other air pollutants present in the air at the same time.

Acknowledgement

REFERENCES

Adams, W.W. and Demmig-Adams, B. 1992. Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. *Planta* **186**: 390–398.

Alstrup, V. 1992. Effects of pesticides on lichens. *Bryonora* **9**: 2–4.

Bartók, K. 1999. Pesticide usage and epiphytic lichen diversity in Romanian orchards. *Lichenologist* **31**: 21–25.

Benfield, B. 1994. Impact of agriculture on epiphytic lichens at Plymtree, East Devon. *Lichenologist* **26**: 91–96.

Bilger, W., Schreiber, U., and Bock, M. 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* **102**: 425–432.

Brown, D.H. 1992. Impact of agriculture on bryophytes and lichens. In: *Bryophytes and Lichens in a Changing Environment*. J.W. Bates and A.M. Farmer, eds. Clarendon Press, Oxford, pp. 259–283.

Brown, D.H. and Hooker, T.N. 1977. The significance of acidic lichen substances in the estimation of chlorophyll and phaeophytin in lichens. *New Phytologist* **78**: 617–624.

Brown, D.H., Standell, C.J., and Miller, J.E. 1995. Effects of agricultural chemicals on lichens. *Cryptogamic Botany* **5**: 220–223.

Calatayud, A., Sanz, M.J., Calvo, E., Barreno, E., and Del Valle-Tascon, S. 1996. Chlorophyll a fluorescence and chlorophyll content in *Parmelia quercina* thalli from a polluted region of northern Castellon (Spain). *Lichenologist* **28**: 49–65.

Culberson, C.F. and Ahmadjian, V. 1980. Artificial reestablishment of lichens. *Mycologia* **72**: 90–109.

DaSilva, E.J., Henriksson, L.E., and Henriksson, E. 1975. Effect of pesticides on blue-green algae and nitrogen-fixation. *Archives of Environmental Contamination and Toxicology* **3**: 193–204.

Demmig, B., Winter, K., Krüger, A., and Czygan, F.C. 1988. Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. *Plant Physiology* **87**: 17–24.

Demmig-Adams, B. and Adams, W.W. 1994. Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. *Physiologia Plantarum* **92**: 451–458.

Demmig-Adams, B. and Adams, W.W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science* **1**: 21–26.

Farrar, J.F. and Smith, D.C. 1976. Ecological physiology of the lichen *Hypogymnia physodes*. *New Phytologist* **77**: 115–125.

Gauslaa, Y., Kopperud, C., and Solhaug, K.A. 1996. Optimal quantum yield of photosystem II and chlorophyll degradation of *Lobaria pulmonaria* in relation to pH. *Lichenologist* **28**: 267–278.

Hawksworth, D.L. and Rose, F. 1976. *Lichens as Pollution Monitors*. Edward Arnold, London, p. 59.

Hällbom, L. and Bergman, B. 1979. Influence of certain herbicides and a forest fertilizer on the nitrogen fixation by the lichen *Peltigera praetextata*. *Oecologia* **40**: 19–27.

Jensen, M., Roosen, B., and Kuffer, M. 1993. Quantification of the xanthophyll cycle carotenoids in lichens by HPLC. *Bibliotheca Lichenologica* **53**: 109–114.

Jensen, M. 1994. Assessment of lichen vitality by the chlorophyll fluorescence parameter Fv/Fm. *Cryptogamic Botany* **4**: 187–192.

Jensen, M., Linke, K., Dickhäuser, A., and Feige, G.B. 1999. The effect of agronomic photosystem-II herbicides on lichens. *Lichenologist* **31**: 95–103.

Kershaw, K.A. 1985. Photosynthetic capacity changes in lichens and their potential ecological significance. In: *Lichen Physiology and Cell Biology*. D.H. Brown, ed. Plenum Press, New York, pp. 93–109.

Kranner, I. and Grill, D. 1994. Rapid changes of the glutathione status and the enzymes involved in the reduction of glutathione-disulfide during the initial stage of wetting of lichens. *Cryptogamic Botany* **4**: 203–206.

Lichtenthaler, H.K. 1988. *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. In: *Applications of Chlorophyll Fluorescence*. H.K. Lichtenthaler, ed. Kluwer Academic Publishers, Dordrecht, pp. 129–142.

Loppi, S. and De Dominicis, V. 1996. Effects of agriculture on epiphytic lichen vegetation in central Italy. *Israel Journal of Plant Sciences* **44**: 297–307.

Modenesi, P. 1993. An SEM study of injury symptoms in *Parmotrema reticulatum* treated with paraquat or growing in sulphur dioxide-polluted air. *Lichenologist* **25**: 423–433.

Pfeifhofer, H.W. 1989. On the content of Norway spruce needles infected with *Chrysomyxa rhododendri* and the carotenoids of fungus aeciospores. *European Journal for Pathology* **19**: 363–369.

Piervittori, R., Usai, L., Alessio, F., and Maffei, M. 1997. The effect of simulated acid rain on surface morphology and n-alkane composition of *Pseudevernia furfuracea*. *Lichenologist* **29**: 191–198.

Richardson, D.H.S. 1988. Understanding the pollution sensitivity of lichens. *Botanical Journal of the Linnean Society* **96**: 31–43.

Ruoss, E. 1999. How agriculture affects lichen vegetation in central Switzerland. *Lichenologist* **31**: 63–73.

Seaton, G.G.R. and Walker, D.A. 1990. Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. *Proceedings of the Royal Society, London B* **242**: 29–35.

Seel, W.E., Baker, N.R., and Lee, J.A. 1992. Analysis of the decrease in photosynthesis on desiccation of mosses from xeric and hydric environments. *Physiologia Plantarum* **86**: 451–458.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A., and Galun, M. 1996. Comparative studies on *Xanthoria parietina*, a pollution-resistant lichen, and *Ramalina duriaei*, a sensitive species. I. Effects of air pollution on physiological processes. *Lichenologist* **28**: 355–365.

Silberstein, L., Siegel, B.Z., Siegel, S.M., Mukhtar, A., and Galun, M. 1996. Comparative studies on *Xanthoria parietina*, a pollution-resistant lichen, and *Ramalina duriaei*, a sensitive species. II. Evaluation of possible air pollution-protection mechanisms. *Lichenologist* **28**: 367–383.

Shaaltiel, Y., Glazer, A., Bocion, P.F., and Gressel, J. 1988. Cross tolerance to herbicidal and environmental oxidants of plant biotypes tolerant to paraquat, sulfur dioxide and ozone. *Pesticide Biochemistry and Physiology* **31**: 13–23.

Smith, D.C. and Molesworth, S. 1973. Lichen physiology. XIII. Effects of rewetting dry lichens. *New Phytologist* **72**: 525–533.

Sonesson, M., Callaghan, T.V., and Börns, L.O. 1995. Short-term effects of enhanced UV-B and CO_2 on lichens at different latitudes. *Lichenologist* **27**: 547–557.

Thiele, A., Krause, G.H., and Winter, K. 1998. *In situ* study of photoinhibition of photosynthesis and xanthophyll cycle activity in plants growing in natural gaps of the tropical forest. *Australian Journal of Plant Physiology* **25**: 189–195.

Tretiach, M. and Carpanelli, A. 1992. Chlorophyll content and morphology as factors influencing the photosynthetic rate of *Parmelia caperata*. *Lichenologist* **24**: 81–90.

Vagts, I., Kinder, M., and Müller, J. 1994. The effect of agrochemicals on the growth of *Cladonia furcata*. *Lichenologist* **26**: 73–82.

Van Herk, C.M. 1999. Mapping of ammonia pollution with epiphytic lichens in the Netherlands. *Lichenologist* **31**: 9–20.

Young, A. and Britton, G. 1990. Carotenoids and stress. In: *Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. R.G. Alscher and J.R. Cumming, eds. Wiley& Sons, New York, pp. 87–112.