Archives of Environmental Contamination and Toxicology (2023) 84:73–84
https://doi.org/10.1007/s00244-022-00973-0

Simulation of Drift Depositional Rate of the Fungicide Fosetyl and Its Effects on Non-vascular Plants: Study Case of the Epiphytic Lichen *Pseudevernia furfuracea*

Anna Corapi[1] · Luana Gallo[1] · Lucio Lucadamo[1]

Received: 27 July 2022 / Accepted: 14 December 2022 / Published online: 25 December 2022

Abstract

The epiphytic lichen *Pseudevernia furfuracea* was exposed to a simulation of drift deposition rate of the fungicide Fosetyl-Al in an indoor controlled environment by testing two exposure factors: pesticide concentrations (based on the application rates of 4 kg ha^{-1} and 1.6 kg ha^{-1}) and drop sizes (anti-drift nozzle: 386–484 µm; non-anti-drift nozzle: 159–231 µm) for a total of four treatments. Drift for higher application rate was simulated once and that for the lower one twice to reproduce agricultural practices. Following fungicide spraying, we measured the concentration of Fosetyl and phosphonic acid in lichen thalli, and the response of ecophysiological status parameters. No trace of Fosetyl was quantifiable 4 days after each treatment, being detected only phosphonic acid whose concentrations stayed substantially unchanged for the whole duration of the experiment (40 days) and resulting affected by application rate and not by drop size. Both pesticide concentrations caused a remarkable harmful effect that, however, was statistically significant vs control group only starting from the 20th day of stay in the climatic chamber. The drift associated with the higher rate resulted, on average, to be 83% more effective, with the most affected parameters being membrane integrity, lipid peroxidation and photosynthetic pigments. Because the selected lower rate can be considered a quite low value when compared with the rank of used rates for crop protection, the Fosetyl-Al formulate is classifiable as hazardous for its effect on non-target organisms.

Industrial agriculture reduces the loss in crop yield due to a wide range of strongly competing native vegetation, insects and phytopathogens by systematically spraying biocide active substances which, when drifting out of the targeted zone, result in possible ecosystems contamination and harmful consequences to human health (Kumar Yadav 2010; Linhart et al. 2019). Many factors contribute to the aerial dispersion of pesticides, i.e., boom height, nozzle types, spray pressures, carrier volume, wind speed, air stability (Ozkan and Zhu 2016; Hofman and Solseng 2017). The combination of these variables results in very high potential contamination scenarios with drift affecting from an extremely low amount of sprayed formulation to percentage approaching more than half of the volume used for application (Kehoe 2012; Ellis et al. 2002). The area outside of the agricultural field where pesticide deposition takes place is likewise strongly variable. However, length of trajectories can range from a few meters to tens/hundreds meters as well as kilometers (Cunha da 2008; Holterman et al. 1997; Ward et al. 2006; Park et al. 2002; Kruger et al. 2019; Lucadamo et al. 2018). Organophosphates are the most used insecticides and widely applied for weed controls with an average global annual utilization of 69,571 metric tons whose test contribution is due to Asia (39%) and America (38%) (Stecker 2018). Fosetyl is one of the most frequently organophosphate fungicide applied in Europe, Asia, Australia and USA. Its detrimental effects on aquatic and terrestrial organisms has been studied and detected (Barreto et al. 2021; Geret et al. 2011; Pilbeam et al. 2000), whereas there is no substantial conclusion about the potential toxic effect to human beings (EFSA 2005). Non-vascular plants contribute around 10% to total Net Primary Productivity (Whittaker 1996); however, in some ecosystems, covering more than 15% of global land surfaces (Druel et al. 2017), due to the high nitrogen recycling (Mekkonen et al. 2018), their contribution can raise to 25–50% (Beringer et al. 2001; Campioli et al. 2009). In addition, mosses and lichens are the most used biomonitors for evaluation of the air quality, but despite these premises, few

✉ Anna Corapi
anna.corapi@unical.it

[1] Department of Biology, Ecology and Earth Sciences, University of Calabria, 87036 Arcavacata di Rende, CS, Italy

studies were accomplished to investigate the toxicological and ecological effects of pesticide drift on these organisms (Lucadamo et al. 2018; Vannini et al. 2015; McCullin et al. 2012; Newmaster et al. 1999). Based on the above-mentioned considerations, an experimental design was set up to test the potential negative consequences of a Fosetyl-Al drift simulation on the epiphytic lichen *Pseudevernia furfuracea* depending on different application rates and dimension of nozzles used to spray formulate solutions. Work specifically was aimed at: (a) testing the effect, on Fosetyl bioaccumulation, of drift simulations resulting from two typical agricultural fungicide rates (Low Rate = B, and High Rate = A), and two different types of nozzles, i.e., NADN (Non-Anti-Drift Nozzle) and ADN (Anti-Drift Nozzles) used for fungicide applications, (b) evaluating the conversion of Fosetyl to phosphonic acid depending on sampling dates, (c) verifying the development of alterations of lichen ecophysiological parameters following the four combinations of Rate x Nozzle comparing treated with not treated lichens, in each of the sampling dates, d) evaluating the association between variation of lichen physiological status and that of Fosetyl/phosphonic acid concentrations.

Materials and Methods

Lichens Collection and Acclimation

Lichen thalli were collected from a source area, La Fossiata (Sila National Park, Calabria region) where they show a very intense colonization on coniferous trees and whose annual mean values of temperature and humidity are, respectively, 10 °C and 74%. Once transported to the laboratory of Ecology and Ecotoxicology of Department of Biology, Ecology and Earth Sciences (University of Calabria), lichens were first completely detached from branches and twigs and then both pieces of bark and microfauna were carefully removed. Eighty 6-g samples of lichens were weighed by an analytical balance (Sartorius R200D) and housed within as many squared (15 cm × 15 cm) top opened plastic boxes, locating thalli on 1-cm mesh net suspended at a height of ten centimeters by appropriate plastic hooks. Boxes were introduced in an acclimatized chamber (conditions: temperature 18 °C, humidity 60%, illumination 9000 lx light/dark 12 h cycle), placed on a perfect flat surface in a completely randomized arrangement and acclimatized for 5 days before starting treatments.

Drift Simulation

The fungicide commercial formulation used for drift simulation consisted of the water dispersion granules with 80% Fosetyl-Al. The simulation of the contamination process was intended in estimating and reproducing the Fosetyl-Al drift deposition rate. Two application rates for prevention of pathogenic fungal infections were selected: 5 kg ha^{-1} (citrus fruits) and 2 kg ha^{-1} (pome fruits). Drift was supposed to involve 15% of the rates for a total export, respectively, of 600 g and 240 g Fosetyl-Al. The area potentially affected by drift process was calculated based, for one side, on a 100-m length (pesticide application rate referring to 1 hectare = 100 m × 100 m), and for the other supposing, a maximum deposition distance of 15 m that was considered a reasonable scenario between closest and furthest deposition trajectories (see above references). No attempt to simulate a distance concentration gradient was made assuming most of the drift deposition processes takes place within the above mentioned strip of land and intending such a deposition as an "average rate." Indeed, the practice of reproducing drift by means progressively diluted pesticide solutions is not correct because in the real situations, the concentrations of drops does not change or sometimes increase (due to evaporation) with distance from the spraying zone (Cederlund 2017; Roider et al. 2008). The boxes have a surface of 225 cm^2, as a consequence, theoretically, for each gram of lichen biomass, the treatments should result in deposition of respectively (5 kg ha^{-1}) 1875 µg fungicide/1500 µg Fosetyl-Al (Rate A) and (2 kg ha^{-1}) 750 µg fungicide/600 µg Fosetyl-Al. Solutions of 5 g L^{-1} (Rate A) and 2.5 g L^{-1} (Rate B) were prepared, considering that the carrier volume used for field application are 1250 L ha^{-1} and 1000 L ha^{-1}. Spraying of solutions was carried out by means a piston pump (Volpi) in stainless steel with ball valve powered by a lithium battery and two flat fan nozzles: AXI-ISO/110 015 generating a drop size range of 159–231 µm (NADN = Non-Anti-Drift Nozzles) and AVI-ISO/110 015 producing drop sizes ranging from 386 to 484 µm (ADN = Anti-Drift Nozzles). Fungicides were sprayed at 60 cm away from the lichen biomass surface. Because the aim of the work was to reproduce the environmental effects of current agricultural practices, the Rate A was applied once (prevention from *Phytophthora* spp.) and Rate B twice two weeks apart (prevention from *Venturia* spp.). Overall, lichens were subjected to four treatments: Rate A-NADN, Rate A-ADN, Rate B-NADN, Rate B-ADN with a replication level equals to 4. Four sampling dates were provided to analyze the evolution of fungicide bioaccumulation/conversion in phosphonic acid and physiological status damage so that 16 boxes were sprayed for each treatment and 16 more were sprayed only with water (controls), i.e., an independent-randomized sampling design (Table 1, Fig. S1).

Fungicide Analysis

The determination of Fosetyl and phosphonic acid concentrations were performed by Agro.Biolab Laboratory S.r.l., Rutigliano (Bari). Lichen thalli were processed according

Table 1 Experimental design: 6-g lichen thalli amount of *Pseudevernia furfuracea* housed in plastic boxes (n° 80) located in a climatic chamber

Climatic Chamber conditions for the 40 days experimental duration: temperature 18 °C, humidity 60%, illumination 9000 lx (12 h light/dark cycle)					
Lichen conditioning to the environment of the chamber: 5 days					
Nozzles	Rate A: 5 kg ha^{-1} Fungicide—4 kg ha^{-1} Fosetyl-Al. 15% Drift: 750 g Fungicide -600 g Fosetyl-Al		Rate B: 2.5 kg ha^{-1} Fungicide—1.6 kg ha^{-1} Fosetyl-Al. 15% Drift: 300 g Fungicide—240 g Fosetyl-Al		Controls (only water)
	ADN	NADN	ADN	NADN	
Day 6: Fungicide Spraying	16 boxes	16 boxes	16 boxes	16 boxes	16 boxes
Day 10: Fosetyl/Phosphonic acid concentration and physiological status determination	4 boxes	4 boxes	4 boxes	4 boxes	4 boxes
Day 20: Phosphonic acid and physiological status determination	4 boxes	4 boxes	4 boxes	4 boxes	4 boxes
Day 20: Second application of Rate B			16 boxes	16 boxes	4 boxes
Day 24: Phosphonic acid and physiological status determination			4 boxes	4 boxes	4 boxes
Day 30: Phosphonic acid and physiological status determination	4 boxes	4 boxes			4 boxes
Day 40: Phosphonic acid and physiological status determination	4 boxes	4 boxes	4 boxes	4 boxes	4 boxes

In all sampling dates, a set of four boxes for each treatment (controls included) was collected for analysis of Fosetyl/Phosphonic acid and ecophysiological parameters

to the EURL-SRM QuPPe-PO analytical procedure (2017) to evaluate separately the bioaccumulation of Fosetyl and phosphonic acid. When the former was below the quantification limit of method the concentration was calculated by application of 1.34 conversion factor (EFSA 2021). An aliquot of 0.5 g of lichen thalli was weighed in a 50-mL centrifuge tube. Internal standard ($^{18}O_3$ phosphonic acid—CVUA—Chemisches und Veterinäruntersuchungsamt, Stuttgart) was added together with water until a final weight of 10 g. Sample was vigorously shaken by hand for 1 min and left to rest for 10 min to make possible its complete humidification. Then, 10 mL of methanol acidified with 1% formic acid was added, followed by a 20-min tough mechanical stirring and centrifugation per 5 min at 2500 g. Surfactant was filtered by 0.2 µm cellulose filter and 1 mL transferred into an autosampler vial of an LC/LC–MS apparatus. The instrumental configuration was the following: 2 Shimadzu Liquid Chromatograph Nexera X2 LC30AD pumps, Column oven Prominence Shimadzu CTO-20AC, Autosampler Shimadzu SIL 30AC, Degassing unit Shimadzu DGU 20A 5R, column Hypercarb 2.1 × 100 mm 5 µm (P/N 35005–102130), detector AB Sciex LC/MS/MS Triple Quad 5500 QTRAP. Chromatograph analysis was performed based on an electrospray ionization negative mode, at 40 °C column temperature, and an elution gradient according to the conditions illustrated in Table S1.

Because the investigated substances were below the detection limits in not treated lichens, five samples were added with as many increasing concentrations of the analytes that were processed as the other samples and used as analytical standards for calculation of calibration line whose equation was: $y = 0.80316\times$ ($r = 0.99998$). Table S2 shows the matrix concentrations used for calibration and accuracy of measurements. Detection and quantification limits resulted, respectively, to be: 0.020 mg Kg^{-1} and 0.100 mg Kg^{-1}. The results were expressed as µg g^{-1} dry weight of lichen thallus. Samples where no trace of Fosetyl and phosphonic acid were detected, within all analyzed batches, were added with increasing concentrations solutions and underwent to the above-mentioned extraction and quantification procedure, with a matrix recovery ranging between 80 and 120%. Recovery of the internal standard $^{18}O_3$ phosphonic acid was 98%.

Ecophysiological Parameters

Electrical Conductivity

Integrity of cell membranes was evaluated according to the methodology of Marques et al. (2005). 100 mg of thallus were repeatedly rinsed in deionized water to remove external particulate until a stable value of conductivity was measured. Subsequently, electrical conductivity was again measured in a 50-mL water volume before and after a 1 h shaking of the lichen sample. Then, the thallus was boiled per 10 min and a final determination of conductivity was performed. The amount of membrane damage was expressed as percentage (EC%) difference between the value measured following 1 h water shaking and boiling after subtraction of the first 50-mL volume measurement of electrical conductivity.

Lipid Peroxidation

Oxidative damage was quantified as thiobarbituric acid reactive substances (TBA rs) levels (Huang et al. 2004). A 50-mg lichen sample was blended by an Ultraturrax homogenizer (T25, IKA, Germany) with 2.5 mL of a 0.1% trichloroacetic (TCA) solution. After centrifugation at 12,000 × g for 20 min, an aliquot of 0.5 mL was added to 1.5 mL of 0.6% TBA in 10% TCA, and the resulting mixture was incubated at 95 °C for half an hour. At the end of the reaction, the mix was cooled and centrifuged again at 12,000 × g for 10 min. Spectrophotometric analysis (Spectrophotometer Lambda 40, PerkinElmer) was performed on the supernatant and TBArs amounts were quantified taking readings at 532 nm using the molar extinction coefficient (155 mM^{-1} cm^{-1}) of the adduct MDA (Malondialdehyde)—TBA.

Lichen Vitality

The assay evaluates the dehydrogenases activity by means measurements of the enzymatic reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) (Bačkor and Fahselt 2005). Fifteen mg of thallus was incubated at 25 °C for 20 min in a 0.05 M phosphate buffer (pH 6.8) containing 0.6% TTC and 0.005% Triton X-100. The TPF was first sequentially extracted with dimethyl sulfoxide (DMSO) and n-hexane and then quantified, as absorbance units at 492 nm by means of a Spectrophotometer (Lambda 40, PerkinElmer).

Photosynthetic Pigments

Thalli were first pre-treated with $CaCO_3$ saturated acetone to remove lichenic substances. 60 mg of lichen was homogenized with an Ultraturrax (T25, IKA, Germany) in 3 mL of DMSO containing 3 mg of polyvinylpolypyrrolidone and incubated for 18 h in the dark at room temperature. Then, the suspension was centrifuged at 4000 rpm for 10 min, and 3 mL of DMSO was added to the precipitate which was stored for 6 h in the dark at room temperature. The two extraction volumes of DMSO were again centrifuged at 4000 rpm for 10 min and mixed. The absorbance of supernatant was read at 665, 649, 480, 435 and 415 nm with a PerkinElmer λ40 Spectrophotometer. Pigments concentrations were calculated according to the equations of Wellburn (1994). The phaeophytization coefficient was expressed as the ratio of the absorbance at 435 and 415 nm (Ronen and Galun 1984).

Photosynthetic Efficiency

This parameter estimates the photochemical efficiency of Photosystem II and was measured by means of the Handy PEA chlorophyll fluorimeter (Hansatech Instruments Ltd.) (Jensen 2002). After hydration, thalli were kept at room temperature for 1 h. Then, they were dark adapted for 10 min followed by two fluorescence measurements, first immediately after exposure to ambient light (F0) and then after exposure to saturating light (2400 µmol s^{-1} m^{-2}) (Fm). Fv was calculated subtracting F0 from Fm, and the photosynthetic efficiency was expressed as the ratio Fv/Fm.

Statistical Analysis

Fosetyl was determined by pooling four samples for each combination of sampling date × treatment for a total of 21 measurements, controls group being analyzed on 5 dates (see Table 1). The official methods of EFSA provide an analytical procedure for fungi (Anastassiades et al. 2021) but not for lichens, a matrix much more complex. Following preliminary discussions with the certified laboratory commissioned to perform the Fosetyl determination, we sent to it, per each replicate, more than the double of the biomass requested for fungi analysis. Nevertheless, it resulted not sufficient for an accurate measurement of fungicide concentration. As a consequence, determination of Fosetyl was performed after pooling the four replicates for each combination of application rate × nozzle. Two separate statistical approaches were used to evaluate the effect of application rate and nozzles on fungicide bioaccumulation. A test on frequencies (a Goodness-fit Chi-square) was performed to test the effect of the four combinations (application rate × nozzle) by preliminary calculation of the ratio (per application date) between detected and expected (if all the sprayed fungicide deposited on treated thalli surface) bioaccumulated amounts of fungicide. Lack of falsification ($p > 0.05$), i.e., all 4 ratios non-statistically different from 1 meaning no differences in effect between treatments on Fosetyl bioaccumulation. A Mann–Whitney test was performed to test, separately, the effect on bioaccumulation of application rate and nozzle with a replication level equals to 8.

Transformation of Fosetyl into phosphonic acid was evaluated by comparing their single measurement of concentration per each sampling date.

As regard the effects of treatments on ecophysiological parameters, each temporal measurement was not performed on the same thalli but always on a different set of replicates, i.e., a totally random independent design. Two types of non-parametric Anova (Kruskal–Wallis) with post hoc multiple comparison (Nemenyi test) were performed: (a) comparing, separately, each sampling date of the four treatments (NADN-Rate A, NADN-Rate B, ADN-Rate A, ADN-Rate B) with controls to evaluate potential statistical significant differences (evaluation of the effect of the treatments), replication level equals to 4, (b) comparing all treatments

(evaluation of differences in efficacy between treatments per each sampling date), replication level equals to 4.

Association between variation of fungicide bioaccumulation and variation of ecophysiological parameters was tested by performing two nonparametric (Spearman) correlations analyses: (a) between the two whole series of data, replication level equals to 20, and (b) between the same series of data split in two halves, respectively, first two sampling dates and second two sampling dates, replication level equals to 10. In addition, a further correlation was performed between ecophysiological parameters to detect possible interactions between different types of cell damage.

Results

Four days after the treatment of lichens with the fungicide, i.e., days 10th and 24th, no amount of Fosetyl above the quantification limits was detected, being found only its metabolite, phosphonic acid (Fig. 1a). The concentration of Fosetyl, due to the fungicide solution spraying, was calculated based on the 1.34 EFSA (2021) conversion factor (Fig. 1b). Both Fosetyl and acid phosphonic were never detected in controls.

Although some dates showed a relatively unexpected high value, within the same treatment; however, the average coefficient of variation per treatment was 12%, and the detected oddities were probably caused by the high variation in micromorphology of thalli (like bush morphotype imitating a micro-canopy effect) that resulted in uneven distribution of biocide on lichen surface (due to differential local scale trapping capacity of spraying). The results of Goodness Fit Chi Square tests are presented in Tables 2 and 3.

Both tests showed a p value associated with Chi-square > 0.05 supporting the idea that the different nozzles did not substantially affect, for each rate of fungicide application, the total input of Fosetyl settled on the 6 g of lichen biomass. The calculated "expected" value of Chi-square suggests that fungicide spraying resulted in a slight overloading of exposed lichens (between 7.4 and 15% higher than 1). The same indication is given by the

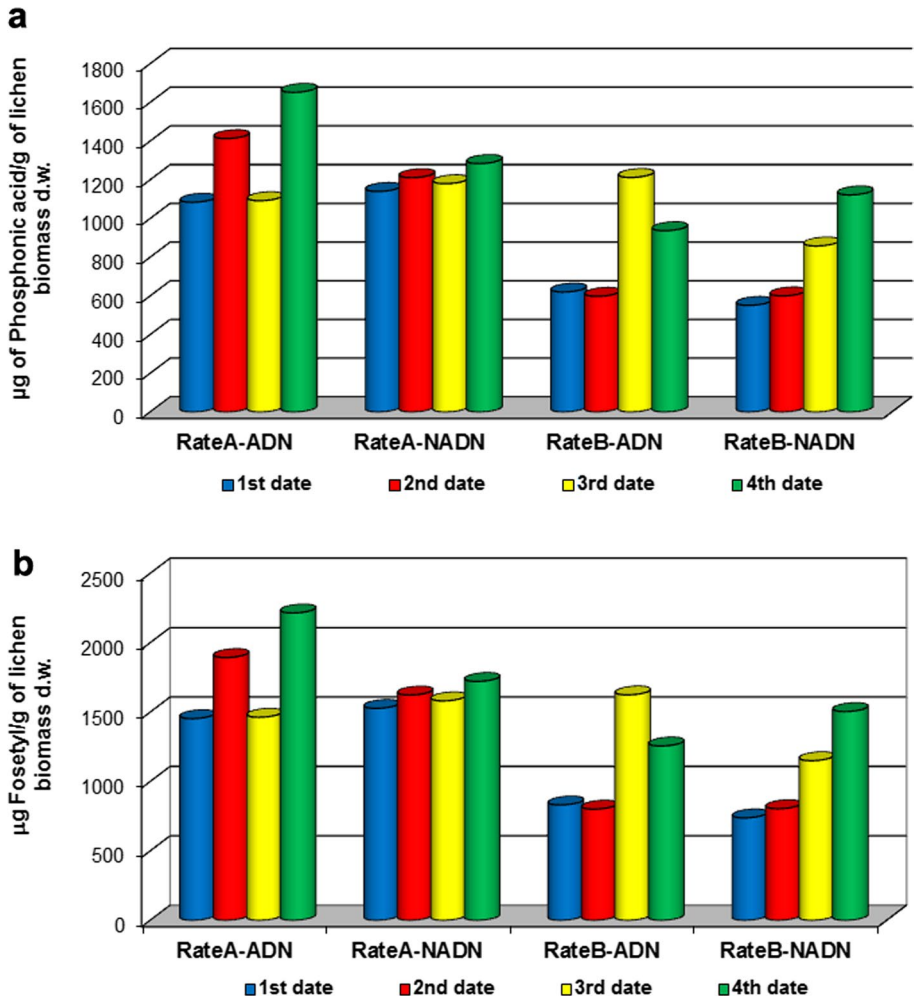

Fig. 1 Concentrations of phosphonic acid **a** and Fosetyl **b** detected in lichen thalli depending on the four treatments applied. NADN = Non-Anti-drift nozzles, ADN = Anti-drift nozzles

Table 2 Chi-square test performed on the ratio between the per date (4 samples pooling) concentration of Fosetyl following spraying and the theoretical expected load of Fosetyl settled on the lichens based on the four treatments

Test contribution				
Category	Observed	Proportion	Expected	Chi square
Rate A-ADN	0.968	0.25	1.15	0.028
Rate A-NADN	1.018	0.25	1.15	0.015
Rate B-ADN	1.385	0.25	1.15	0.047
Rate B-NADN	1.230	0.25	1.15	0.005
N	d.f	Chi-square		*p*
4.601	3	0.097		0.992

Comparison related to the treatments applied in day n. 6

Table 3 Chi-square test performed on the ratio between the per date (4 samples pooling) concentration of Fosetyl following spraying and the theoretical expected load of Fosetyl settled on the lichens based on the four treatments

Test contribution				
Category	Observed	Proportion	Expected	Chi square
Rate A-ADN	0.968	0.25	1.074	0.010
Rate A-NADN	1.018	0.25	1.074	0.002
Rate B-ADN	1.353	0.25	1.074	0.072
Rate B-NADN	0.958	0.25	1.074	0.012
N	d.f	Chi-square		*p*
4.298	3	0.098		0.992

Comparison related to the treatments applied in day n. 6 Rate A-ADN, Rate A-NADN and 20 Rate B-ADN, Rate B-NADN

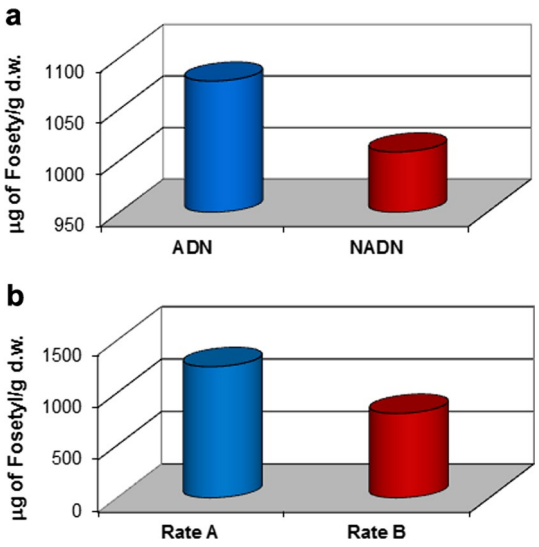

Fig. 2 Medians of Fosetyl concentrations calculated based on the different nozzles (ADN and NADN) **a** and on the different application rates (Rate A: 4 kg ha^{-1} and Rate B: 1.6 kg ha^{-1}) **b**

comparison (Fig. 2) of values of Fosetyl averaged respectively per Nozzles (ADN-NADN) and application rate (Rate A and Rate B).

The Mann–Whitney test performed on the two comparison showed a significant result for application rate ($W = 93$, $p = 0.010$) and not significant for nozzles ($W = 71$, $p = 0.793$) again supporting the idea that the two ways of spraying fungicide solution did not result in different total load of Fosetyl settled on the lichen thalli. Table S3 shows the central tendency values, per sampling date, of the ecophysiological parameters in relation to the four treatments, as well as the general average of Control group (due to lack of statistical differences between the 5 dates).

All the post hoc comparisons (Nemenyi test), following Kruskal–Wallis, of treatments with control group showing a value higher than the MSD ($p < 0.05$) are detectable in the third and fourth sampling dates suggesting a temporal trend in the development of biological stress. When

evaluating the potential effect of treatment rates and nozzles on these results, it is evident that if the comparisons statistically significant are merged according the different nozzles 46.7% are associated with the category NADN and 53.3% to that ADN, whereas if the same merging is accomplished according the rate a clear distinction is detectable: Rate A 63.4% and Rate B 36.6% (incidence 4.7 times higher). However, interestingly if a Mann–Whitney test is performed factorizing for Drop Size and Rate, 1 statistical significant difference is detected in the first case for TBArs (ADN > NADN), while three statistical significant differences are detected in the second case for TBArs, Fv/Fm and EC% (Rate A > Rate B). Table 4 illustrates the average percentage increase vs control of the treatment rates.

The Rate A increase is, on average, 83% higher than that of Rate B (52.8% vs 28.8%) with EC%, TBArs and Fv/Fm showing the highest differences. On the contrary, the gaps of pigments are virtually the same. The covariance respectively between ecophysiological parameter pairs and the phosphonic acid concentrations again with mycobiont and photobiont physiological status parameters are illustrated in Tables S4 and S5.

Only oxidative damage and the membrane integrity show to covariate with most of the other ecophysiological parameters supporting a driving role of the former in affecting the change in the physiological status of lichens once compared to the control. On the other hand, the variation of phosphonic acid, along the whole experimental duration, strongly associates with the variation of seven out of ten parameters and shows the highest correlation just with the TBArs and EC%. Interestingly, when the dataset is split in two halves, the number of significant correlations reduces drastically in the first emi-series (where no significant difference with control is detected), while stays unchanged in the second emi-series (where all the statistically significant variations vs control are detectable).

Table 4 Differences in average physiological status parameters of the two treatment rates compared to control expressed as percentage variation

Ecophysiological parameters	Rate B	Rate A
EC%	61.41	204.82
TBArs	49.45	87.59
Chlorophyll a	−48.61	−48.52
Chlorophyll b	−41.41	−45.34
Xanthophylls + Carotenoids	−39.86	−42.77
Fv/Fm	−16.37	−42.15
A492	−11.31	−16.23
A492 g^{-1} d.w	−11.16	−14.97
OD435/OD415	−14.89	−12.99
OD435/OD415 g^{-1} d.w	−14.66	−12.87

Discussion

The scientific literature dealing with volume median diameter show contradictory results about performance in field and plant coverage, and however, never in relation to simulations of drift depositional rate. Small droplets may be more effective in crop surface coverage and retention (Wolf et al. 2000, 2009) as well as in treatment efficacy (Cross and Barrie 1995; Prasad 1992), but increasing carrier volume can result in a compensating effect making big droplets better performing in surface covering (Bretthauer et al. 2008; Butts et al. 2018) and grass species control (Ramsdale 2001a; b; Douglas 1968). In the present work, we calculated and simulated the deposition rate of Fosetyl drift events. No difference was detected depending on the use of anti-drift nozzles vs non-anti-drift nozzles, in relation to the concentration of fungicide measured in lichen biomass exposed to the biocide spraying. According to our opinion, this result was due to the presence of two co-formulants, the ethoxylated-propoxylated fatty alcohol (1–3%) and the lignosulfonic acid (3–10%) both dispersant agents (ECHA 2021; De Ruiteret al. 2003) needed to improve the solubilization of the Fosetyl wettable powder. These molecules promote a better equidistribution of the fungicide between the droplets so that this process compensates enough for the amount of carrier not delivered to the box surface because of the high dispersion affecting the low size droplets (CSIRO 2002; CIPM 2022) compared to very coarse droplet treatments (Carroll 2017). This outcome suggests the crucial role that adjuvants can play in modifying the amount of active ingredient lost during pesticide application increasing efficiency of treatments (Preftakes et al. 2019; Curran et al. 1999; Griesang et al. 2017). No trace of Fosetyl was detected 4 days after the application. Several literature data indicate that metabolism of this molecule is quite fast. Indeed, within 12–24 h from the application, phosphonic acid is found within tissues of plants treated with the fungicide (Pelegri et al. 1993; Fenn 1986). However, several field trials suggested that some cultivars can show appreciable amount of Fosetyl also long enough after spraying (Bellisai et al. 2021; Dann and McLeod 2020). This leads to the conclusion that rate of degradation of Fosetyl is species-specific and, at the moment, no work exists dealing with this process in non-vascular plants although the official analytical methods for Fosetyl detection provides a procedure for fungi (Anastassiades et al. 2021). Lichens can uptake both inorganic and organic forms of phosphorous and Fosetyl molecule can act as a trophic resource. First of all, the "P" metabolismis quite efficient, indeed lichens need 1 h of rainfall per week, based on the average year

phosphorous concentrations in bulk depositions, to satisfy their request (Farrar 1976). The mineralization of organic form of phosphorous takes place by means several mono- and diphosphorus esterase located in the cytoplasm, cell wall and junctions of fungal partner detectable both in fruticose lichens like *Cladonia portentosa* (Hogan 2003) and in foliose ones like genus *Peltigera* (Stevenson 1994). Once converted in an inorganic anion, phosphorous is fastly carried inside the mycobiont cells (Farrar 1976) or translocated to the photobiont cells (Kono et al. 2020), through active transport mechanisms, as suggested by Smith (1973) that showed that the lichen *Peltigera polydactila* can uptake as much mineral phosphorous as an equivalent of 3% of its dry weight in 24 h. Overall such a scenario is compatible with our results making us hypothesize that in *P. furfuracea* the degradation of Fosetyl as well as mineral phosphorous absorption are both processes performed very effectively. When considering the response of *P. furfuracea* to the exposure to the 4 treatments, again the difference in drop size does not seem to affect particularly the change in symbionts physiology except for oxidative damage may be as a consequence of higher amount of pesticide carried by each single big drop. Perhaps this outcome is due to the fact that big drops may result in a more "localized" damage associated with the higher amount of per single drop (Feng et al. 2003; Hanna et al. 2009). The remarkable physiological changes detected in thalli are mostly due to different rates. This is clearly indicated by: (a) three times higher number of statistically significant differences vs control detected comparing Rate a with Rate B rather than ADN and NADN, (b) the higher number of parameters (3) affected by different Rates compared to different nozzles (1) when considering total means. However, the overall greater effect on lichen physiological status is mainly attributable to the difference in rates. Indeed, it is clearly indicated from: (a) the strong segregation between Rate A and Rate B in percentage cases significantly higher than controls in the 3rd and 4th sampling dates vs drop sizes, (b) the lower number of ecophysiological parameters that significantly differ between the two drop sizes i.e. just TBArs, compared to the different Rates, i.e., TBArs, Fv/Fm and EC% when the "total" means are taken into consideration. The latter are also those showing the strongest difference both between the two rates (Rate A > Rate B) and between Rate A and control group with the rupture of membrane being the physiological indicator mostly affected. Fosetyl can prevent fungal infection by both indirect mode of action, i.e. promoting plant defense response (Derks and Creasy 1989; Nemesthoty and Guest 1990), and direct mechanisms like parasite dehydrogenases inhibition (Stehmann and Grant 2000) and unbalance of ATP metabolism (Niereet al. 1994; Griffith et al. 1990).

The analysis of correlation between ecophysiological parameters suggests that, in our experiment, osmotic stress mechanisms seem to play a crucial role in driving cell impairment (Rezende 2020; Abertonet al. 1999) that triggers an increase in oxidative damage. Indeed, the loss of membrane integrity associated with redox systems like in mitochondria and thylakoids, causes electron dissipation resulting in ROS formation (Qamer et al. 2021; Apel and Hirt 2004), that negatively affects plants, photosystems by means decrease in chlorophylls and carotenoids levels (Havaux 2013; Yamauchi 2015), reduction in chlorophyll a biosynthesis (Aarti et al. 2006) and an impairment of photosynthetic efficiency (Phetchuay et al. 2019). The causative association between variation of ecophysiological parameters and Fosetyl metabolite through the four treatments is strongly strengthened by the high number of statistically significant correlation coefficients. Such an outcome is confirmed by other authors findings showing that as well osmotic stress can cause leaf burn in plants (McCauley and Jones 2005; Wulandari et al. 2021) as crops and natural vegetation exhibit such a foliar damage (Walker 1989; Guest and Grant 1991) following Fosetyl exposure while algae suffer a damage to chlorophyll as denoted by a reduction in growth (Ma et al. 2002; Jianvi et al. 2011). A critical point emerged from our experiment is that the cell damage development is a time dependent process. Drift simulations results in a remarkable alteration of photobiont and mycobiont physiological status only between 20 and 30th day of experiment duration, i.e., the cell integrity is preserved until those dates despite the phosphonic acid accumulation. A possible interpretation comes from the mineral phosphorous storage strategies in lichens. This nutrient can be accumulated as polyphosphates granules inside vacuoles, as demonstrated both in lichen species like *Collema leucocarpon* Wilson and *Peltigera dolichorrhiza* and in fungi (Chilvers et al. 1978; Crittenden 1988). Moreover, phosphorous appears to be in a not active osmotic form because its association with calcium. When P metabolism requests its mobilization, polyphosphates are hydrolyzed by phosphodiesterase (Yang et al. 2017). Our analysis failed to detect polyphosphates so we must to hypothesize that phosphonates are accumulated unaltered inside like vacuoles structures associated with calcium or some other counter-cation. When correlation analysis is split in to two series, those related to the "first two dates" shows significant coefficient only with EC% and A492. This suggests that probably a relatively small amount of phosphonates stay in a free form within fungal cytoplasm generating a weak level of osmotic stress. Later, vacuoles can release the accumulated phosphonates due to rupture promoted by autolysis or spherosomes activity (Wilson et al. 1970; Weber et al. 1999) triggering a diffuse osmotic shock, strongly damaging membrane and causing electrons dispersion. Obviously, such a hypothesis needs further exploration and validation, although it seems consistent with the results of the present work.

Conclusions

Fosetyl was an active ingredient used in organic agriculture, as sodium and potassium salt for fertilizer integration, until 2019 when Europe Union banned its application, tolerating a 0.5% detection limit as consequence of an accidental dispersion (EU 1009/2019). Nowadays, its aluminum salt is widely used in America, Europe, Asia and Australia to eradicate many parasite fungi. Our work showed that it could be hazardous to non-vascular plants as lichens. Definitely, the tested species *Pseudevernia furfuracea* showed to suffer strong membrane and oxidative damage, and a remarkable reduction in pigments concentrations once exposed to drift simulations related to both low and high application rates, although the latter caused the highest alterations compared to not treated lichens. Therefore, the tested formulation, based on the applications requested by agriculture practices, can be considered as a "dangerous product," regarding environmental consequences, because the "low" treatment rate used is among the lowest usually applied for crop protection. The molecule considered responsible for such an effect was its metabolite phosphonic acid which resulted to be the only form detected in lichens already four days after fungicide spraying. In addition, the manifestation of damage appeared not before the 20th day of the experiment, probably due to a previous storing, in a not active osmotic form, within fungi vacuoles. The use of different nozzles did not generate appreciable variation both in phosphonates accumulation and degree of physiological status impairment (although a difference was appreciated in case of oxidative damage), a result, we hypothesize, due to the presence of dispersants in commercial formulate. On the whole, this work underlines the need to reconsider the current suggested application rates of the investigated fungicide in the light of the detected significant toxic effect as well as to combine the pesticide treatments with measures aimed at containing its environmental dispersion like careful monitoring of weather condition at time of spraying and introduction of buffer zones and hedges along the field boundaries.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00244-022-00973-0.

Funding The authors did not receive support from any organization for the submitted work.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

References

Aarti PD, Tanaka R, Tanaka A (2006) Effects of oxidative stress on chlorophyll biosynthesis in cucumber (*Cucumis sativus*) cotyledons. Physiol Plant 128(1):186–197. https://doi.org/10.1111/j.1399-3054.2006.00720.x

Aberton MJ, Wilson BA, Cahill DM (1999) The use of potassium phosphonate to control *Phytophthora cinnamon* in native vegetation at Angle sea, Victoria. Australas Plant Path 28:225–234. https://doi.org/10.1071/AP99037

Anastassiades M, Kolberg DI, Eichhorn E, Benkenstein A, Lukačević S, Mack D, Wildgrube C, Sigalov I, Dörk D, Barth A (2021) Quick method for the analysis of highly polar pesticide in food involving extraction with acidified methanol and LC or IC-MS/MS measurement. I. Food of plant origin (QuPPe-PO-Method) EU reference laboratory for pesticides requiring single residue methods (EURL-SRM), Version 12, p 60. https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_QuPPe_PO_V9_3.pdf

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Bačkor M, Fahselt D (2005) Tetrazolium reduction as an indicator of environmental stress in lichens and isolated bionts. Environ Exp Bot 53(2):214–222. https://doi.org/10.1016/j.envexpbot.2004.03.007

Barreto A, Santos J, Amorim MJB, Maria VL (2021) Is the synthetic fungicide fosetyl-Al safe for the ecotoxicological models *Danio rerio* and *Enchytraeus crypticus*? Appl Sci 11(16):7209. https://doi.org/10.3390/app11167209

Bellisai G, Bernasconi G, Brancato A, Carrasco Cabrera L, Ferreira L, Giner G, Greco L, Jarrah S, Kazocina A, Leuschner R, Magrans JO, Miron I, Nave S, Pedersen R, Reich H, Ruocco S, Santos M, Scarlato AP, Theobald A, Vagenende B, Verani A (2021) Reasoned opinion on the joint review of maximum residue levels (MRLs) for fosetyl, disodium phosphonate and potassium phosphonates according to articles 12 and 43 of Regulation (EC) No 396/2005. EFSA J 19(8):6782. https://doi.org/10.2903/j.efsa.2021.6782

Beringer J, Lynch AH, Chapin FS, Mack M, Bonan GB (2001) The representation of arctic soils in the land surface model: The importance of mosses. J Clim 14(15):3324–3335. https://doi.org/10.1175/1520-0442(2001)014%3c3324:TROASI%3e2.0.CO;2

Brettahuer SM, Mueller TA, Derksen RC, Zhu H, Bode LE (2008) The effects of spray application rate and droplet size on application to control soybean rust. ASABE. American Society of Agricultural and Biological Engineers. Paper No. 084219. As ASAE Meeting Presentation. https://elibrary.asabe.org/abstract.asp?aid=25050

Butts TR, Samples CA, Franca LX, Dodds DM, Reynolds DB, Adams JW, Zollinger RK, Howatt KA, Frit BK, Hoffman WC, Kruger G (2018) Spray droplet size and carrier volume effect on dicamba and glufosinate efficacy. Pest Manag Sci 74(9):2020–2029. https://doi.org/10.1002/ps.4913

Campioli M, Samson R, Michelsen A, Jonasson S, Baxter R, Lemeur R (2009) Nonvascular contribution to ecosystem NPP in a subarctic heath during early and late growing season. Plant Ecol 202(1):41–53

Carroll J (2017) The effects of sprayer speed and droplet size on herbicide burn down efficacy. Graduate theses and dissertations retrieved from https://scholarworks.uark.edu/etd/2435

Cederlund H (2017) Effects of spray drift of glyphosate on non-target terrestrial plants - A critical review. Environ Toxicol Chem 36(11):2879–2886. https://doi.org/10.1002/etc.3925

Chilvers GA, Ming-Lee M, Ashford AE (1978) Polyphosphate granules in the fungi of two lichens. New Phytol 81(3):571–574

CIPM (2022) Center for integrated pest management. Understanding droplet size – Pesticide Environmental Stewardship. South Dakota cooperative extension service. https://pesticidestewardship.org/pesticide-drift/understanding-droplet-size/

Crittenden PD (1988) Nitrogen relations of mat forming lichens. In: Boddy L, Marchant R, Read DJ (Eds.), Nitrogen, phosphorus and sulphur utilization by fungi. Symposium of the British Mycological Society held at the University of Birmingham, April 1988. Cambridge University Press, New York, pp. 243–268

Cross JV, Berrie AM (1995) Field evaluation of a tunnel sprayer and effects of spray volume at constant drop size on spray deposits and efficacy of disease control on apple. Ann Appl Biol 127(3):521–532. https://doi.org/10.1111/j.1744-7348.1995.tb07609.x

CSIRO PUBLISHING (2002) Spray drift management – principles, strategies and supporting information – primary industries standing committee. PISC (SCARM) Report 82. 150 Oxford Street (PO Box 1139) Collingwood VIC 3066, Australia. ISBN 0 643 06835 X

Cunha ARJP (2008) Pesticide drift simulation under different spray conditions. Cienc Agrotec 32(5):1616–1621. https://doi.org/10.1590/S1413-70542008000500039

Curran WS, McGlamery RA, Liebl RA, Lingenfelter DD (1999) Adjuvants for enhancing herbicide performance. PA Coop Extension Svc, Agronomy Facts 37 Published and copyrighted by Pennsylvania State University. https://library.ndsu.edu/ir/bitstream/handle/10365/3066/1847cu99.pdf?sequence=1&isAllowed=y

Dann E, McLeod A (2020) Phosphonic acid: a long-standing and versatile crop protectant. Pest Manag Sci 77(5):2197–2208. https://doi.org/10.1002/ps.6156

De Ruiter H, Holterman H., Kempenaar C, Mol HGJ, de Vlieger JJ, van de Zande JC (2003) Influence of adjuvants and formulations on the emission of pesticides to the atmosphere – a literature study for the Dutch Research Programme Pesticides and the Environment (DWK-359) theme C-2. Plant Research International BV, Wageningen, Reporty 59: 48. https://edepot.wur.nl/38200

Derks W, Creasy L (1989) Influence of fosetyl-Al on phytoalexin accumulation in the *Plasmospora viticola* grape vine interaction. Physiol Mol Plant Pathol 34(3):189–202. https://doi.org/10.1016/0885-5765(89)90043-X

Druel A, Peylin P, Krinner G, Ciais P, Viovy N, Peregon A, Bastrikov V, Kosykh N, Mironycheva-Tokareva N (2017) Towards a more detailed representation of high-latitude vegetation in the global land surface model Orchidee (ORCH-HL-VEGv1.0). Geosci Model Dev 10(12):4693–4722. https://doi.org/10.5194/gmd-10-4693-2017

Douglas G (1968) The influence of size spray droplets on the herbicidal activity of diquat and paraquat. Weed Res 8(3):205–2012. https://doi.org/10.1111/j.1365-3180.1968.tb01423.x

ECHA (2021) Definitions and functions of co-formulants in biocidal products. European Chemical Agency. Date of agreement 17 February 2021. Classification: public. Telakkakatu 6, FI-00150 Helsinki, P.O. Box 400, FI-00121 Helsinki, Finland. https://webgate.ec.europa.eu/s-circabc/d/d/workspace/SpacesStore/4ff02008-0109-43c3-9f2b-ff1373d5e543/CG-45-2021-03%2520Definitions%2520and%2520functions%2520of%2520co-formulants.pdf

EFSA (2005) Conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl. European Food Safety Authority. EFSA Sci Rep 54:1–79

EFSA (2021) European food safety authority. Modification of the existing maximum residue levels for fosetyl/phosphonic acid in citrus fruits resulting from the use of potassium phosphonates. EFSA J 19(11):6926. https://doi.org/10.2903/j.efsa.2021.6926

Ellis JM, Griffin JL, Jones CA (2002) Effect of carrier volume on corn (*Zea mays*) and Soybean (*Glycine max*) response to simulated drift of glyphosate and glufosinate. Weed Technol 16(3):587–592

EU Regulation 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilizing products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (text with EEA relevance). https://eur-lex.europa.eu/eli/reg/2019/1009/oj

EURL-SRM (2017) European Union reference laboratories – Single resolution mechanism: quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC-or IC-MS/MS Measurement I. Food of plant origin. Version 12 (22.07.2021). https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Farrar BJ (1976) The uptake and metabolism of phosphate by the lichen *Hypogymnia physodes*. New Phytol 77(1):127–134. https://doi.org/10.1111/j.1469-8137.1976.tb01506.x

Feng PCC, Chiu T, Ryerse JS (2003) Droplet size affects glyphosate retention, absorption and trans location in corn. Weed Sci 51(3):443–448. https://doi.org/10.1614/0043-1745(2003)051[0443:DSAGRA]2.0.CO;2

Fenn ME (1986) Evidence for the direct mode of action of fosetyl-aluminum and phosphorous acid. PhD thesis, University of California, Riverside-University Microfilms International, 300N Zeeb road, Ann ASrbor, MI 48106, p 121. https://www.proquest.com/docview/303412315?pq-origsite=gscholar&fromopenview=true

Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY, Samain JF (2011) Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific Oyster *Crassostrea Gigas*. Environ Toxicol 28(12):689–699. https://doi.org/10.1002/tox.20764

Griesang F, Decaro RA, dos Antos CAM, Souza Sanntos E, de Lima Roque NH, de Costa FM (2017) How much do adjuvant and nozzles models reduce the spraying drift? Drift in agricultural spraying. Am J Plant Sci 8(11):2785–2794. https://doi.org/10.4236/ajps.2017.811188

Griffith JM, Smillie RH, Grant BR (1990) Alterations in nucleotide and pyrophosphate levels in *Phytophthora palmivora* following exposure to the antifungal agent potassium phosphonate (phosphate). J Gen Microbiol 136(7):1285–1291. https://doi.org/10.1099/00221287-136-7-1285

Guest D, Grant B (1991) The complex action of phosphonates as antifungal agents. Biol Rev Camb Philos Soc 66(2):159–187. https://doi.org/10.1111/j.1469-185X.1991.tb01139.x

Hanna M, Kruckeburg J, Darr M, Steward B (2009) Nozzle and droplet size effects on pesticide performance and drift. Iowa State University. Integrated crop management conference, pp 49–52

Havaux M (2013) Carotenoid oxidation products as stress signals in plants. Plant J 79(4):597–606. https://doi.org/10.1111/tpj.12386

Hofman V, Solseng E (2017) Reducing spray drift. NDSU extension service-North Dakota State University, Fargo, North Dakota. https://www.ag.ndsu.edu/publications/crops/reducing-spray-drift

Hogan EJ (2003) Nitrogen-phosphorus relationships in lichens. PhD thesis, University of Nottingham, United Kingdom, p 355. http://eprints.nottingham.ac.uk/10906/

Holterman HJ, van de Zande JC, Porskamp HAJ, Huijsmans JFM (1997) Modelling spray drift from boom sprayers. Comput Electron Agr 19:1–22. https://doi.org/10.1016/S0168-1699(97)00018-5

Huang ZA, Jiang DA, Yang Y, Sun YW, Jin SH (2004) Effects of nitrose deficiency on gas exchange, chlorophyll fluorescence, and antioxidantenzymes in leaves of rice plants. Photosynthetica 42:357–364. https://doi.org/10.1023/B:PHOT.0000046153.08935.4c

Jensen M (2002) Measurement of chlorophyll fluorescence in lichens. In: Kranner IC, Beckett RP, Varma AK (eds) Protocols in

lichenology. Springer lab manuals. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-56359-1_9
Jianyi M, Senmiao T, Pinwei W, Jianmeng C (2011) Differential toxicity of agricultural fungicides toward three Cyanobacteria and five green algal species. Asia J Chem 23(2):533–536
Kehoe N (2012) How dangerous is pesticide drift? Sci Am https://www.scientificamerican.com/article/pesticide-drift/
Kono M, Kon Y, Ohmura Y, Satta Y, Teray Y (2020) In vitro resynthesis of lichenization reveals the genetic background as symbiosis-specific fungal-algal interaction in *Usnea hakonensis*. BMC Genomics 21:671. https://doi.org/10.1186/s12864-020-07086-9
Kruger GR, Klein RN, Ogg CL, Vieira BC (2019) Spray drift of pesticides. NebGuide. Nebraska Extension. https://extensionpublications.unl.edu/assets/pdf/g1773.pdf
Kumar Yadav S (2010) Pesticide applications-threat to ecosystems. J Hum Ecol 32(1):37–45. https://doi.org/10.1080/09709274.2010.11906319
Linhart C, Niedrist GH, Nagler M, Nagrani R, Temml V, Bardelli T, Wilhalm T, Riedl A, Zaller JG, Clausing P, Hertoge K (2019) Pesticide contamination and associated risk factors at public playgrounds near intensively managed apple and wine orchards. Environ Sci Eur 31:28. https://doi.org/10.1186/s12302-019-0206-0
Lucadamo L, Corapi A, Gallo L (2018) Evaluation of glyphosate drift and anthropogenic atmospheric trace elements contamination by means of lichen transplants in a southern Italian agricultural district. Air Qual Atmos Hlth 11(3):325–339. https://doi.org/10.1007/s11869-018-0547-7
Ma J, Zheng R, Xu L, Wang S (2002) Differential sensitivity of two green algae, *Scenedesmus obliquus* and *Chlorella pyrenoidosa*, to 12 pesticides. Ecotox Environ Saf 52:57–61. https://doi.org/10.1006/eesa.2002.2146
Marques AP, Freitas MC, Wolterbeek HT, Steinebach OM, Verburg T, De Goeii JJ (2005) Cell-membrane damage and element leaching in transplanted *Parmelia sulcata* lichen related to ambient SO_2, temperature and precipitation. Environ Sci Technol 39(8):2624–2630. https://doi.org/10.1021/es0498888
McCauley A, Jones C (2005) Salinity % sodicity management. Soil and water management-module 2. State Montana University. 4481–2 JAN-2005. https://landresources.montana.edu/swm/documents/SW%202%20updated.pdf
McCullin RT, Bell FW, Newmaster SG (2012) The effects of triclopyr and glyphosate on lichens. Forest Ecol Manag 264:90–97. https://doi.org/10.1016/j.foreco.2011.09.039
Mekkonen ZA, Riley WJ, Grant RF (2018) Accelerated nutrient cycling and increased light competition will lead to 21st century shrub expansion in North American arctic tundra. J Geophys Res 123(5):1683–1701. https://doi.org/10.1029/2017JG004319
Nemesthoty GS, Guest DI (1990) Phytoalexin accumulation, phenylalanine ammonia lyase activity and ethylene biosynthesis in fosetyl-Al treated resistant and susceptible tobacco cultivars by *Phytophtora nicotianae* var. *nicotianae*. Physiol Mol Plant P 37(3):207–219. https://doi.org/10.1016/0885-5765(90)90012-M
Newmaster SG, Bell FW, Vitt DH (1999) The effects of glyphosate and triclopyr on common bryophytes and lichens in northwestern Ontario. Can J for Res 29(7):1101–1111. https://doi.org/10.1139/x99-083
Niere JO, Deangelis G, Grant BR (1994) The effect of phosphonate on the acid-soluble phosphorus components in the genus *Phytophtora*. Microbiology 140(7):1661–1670. https://doi.org/10.1099/13500872-140-7-1661
Ozkan HE, Zhu H (2016) Effect of major variables on drift distances of spray droplets. The Ohio State University – College of Food, Agricultural, and Environmental Sciences. https://ohioline.osu.edu/factsheet/fabe-525
Park JS, Wade TL, Sweet ST (2002) Atmospheric deposition of PAHs, PCBs and organochlorine pesticides to Corpus Christi Bay. Texas Atmos Environ 36(10):1707–1720. https://doi.org/10.1016/S1352-2310(01)00586-6
Pelegri R, Gamon M, Coscolla R, Beltran V, Cunat P (1993) The metabolism of fosetyl-aluminium and the evolution of residue levels in oranges and 67 tangerines. J Pest Sci 39(4):319–323. https://doi.org/10.1002/PS.2780390412
Phetchuay P, Peerakietkhajorn S, Duangpan S, Buapet P (2019) Toxicity effects of copper and zinc on the photosynthetic efficiency and oxidative stress-related Parameters of the green alga *Chlorella vulgaris* Beijerinck. J Fish Environ 43(2):14–26
Pilbeam RA, Colquhoun IJ, Shearer B, Hardy GESJ (2000) Phosphite concentration: its effect on phytotoxicity symptoms and colonisation by *Phytophtora cinnamoni* in three under story species of *Eucalyptus marginata* forest. Austral Plant Pathol 29:86–95. https://doi.org/10.1071/AP00016
Prasad R (1992) Some factors affecting herbicidal activity of glyphosate un relation to adjuvants and droplet size. In: Bode LE, Chasin DE (eds) Pesticide formulations and application systems. American Society for Testing and Materials, Philadelphia, PA, standard technical publication 1112
Preftakes CJ, Schleier JJ, Kruger GR, Weaver DK, Peterson RKD (2019) Effect of insecticide formulation and adjuvant combination on agricultural spray drift. Peer J 7:e7136. https://doi.org/10.7717/peerj.7136
Qamer Z, Chaudhary MT, Du X, Hinze L, Azhar MT (2021) Review of oxidative stress and antioxidative defense mechanisms in Gossypium hirsutum L. in response to extreme abiotic conditions. J Cotton Res 4:9. https://doi.org/10.1186/s42397-021-00086-4.
Ramsdale BR, Messersmith CG (2001a) Drift reducing nozzle effects on herbicide performance. Weed Technol 15(3):453–488. https://doi.org/10.1614/0890-037X(2001)015[0453:DRNEOH]2.0.CO;2
Ramsdale BR, Messersmith CG (2001b) Nozzle, spray volume and adjuvants effects on carfentrazone and imazamoz. Weed Technol 15(3):485–491. https://doi.org/10.1614/0890-037X(2001)015[0485:NSVAAE]2.0.CO;2
Rezende DC, Brandao DFR, Brand SC, Blumer S, Pascholati SF, Mafra NM (2020) Mode of action of potassium phosphate in the growth and development of *Phytophthora nicotinae*, causal agent of gummosis in citrus. Res Soc Dev 9(10):e5369108822. https://doi.org/10.33448/rsd-v9i10.8822
Ronen R, Galun M (1984) Pigment extraction from lichens with dimethyl sulphoxide (DMSO) and estimation of chlorophyll degradation. Environ Exp Bot 24(3):239–245. https://doi.org/10.1016/0098-8472(84)90004-2
Roider CA, Griffin JL, Harrison SA, Jones CA (2008) Carrier volume affects wheat response to simulated glyphosate drift. Weed Technol 22(3):453–458
Smith DC (1973) The lichen symbiosis. Oxford University Press, London
Stecker B (2018) Harmful pesticide phase-out calls come as some countries' use rises. Blomberg Law, Environment and Energy. https://news.bloomberglaw.com/environment-and-energy/harmful-pesticide-phaseout-calls-come-as-some-countries-use-rises
Stehmann C, Grant BR (2000) Inhibition of enzymes of the glycolytic pathway and hexose monophosphate bypass by phosphonate. Pestic Biochem Phys 67(1):13–24. https://doi.org/10.1006/pest.1999.2465
Stevenson PARJ (1994) Surface phosphatase activity of *Peltigera* and *Cladonia* lichens. Theses, Durham University, p 168. http://etheses.dur.ac.uk/5138/
Vannini A, Guarnieri M, Bačkor M, Bilovà LS (2015) Uptake and toxicity of glyphosate in the lichen *Xanthoria parietina* (L.) Th. Fr. Ecotoxicol Environ Saf 122:193–197. https://doi.org/10.1016/j.ecoenv.2015.07.030

Walker GE (1989) Phytotoxicity in mandarins caused by phosphorous acid. Australas Plant Path 18(3):57–59. https://doi.org/10.1071/app9890057

Ward MH, Lubin J, Giglierano J, Colt JS, Wolter C, Bekiroglu N, Camann D, Hartge P, Nuckols JR (2006) Proximity to crops and residential exposure to agricultural herbicides in Iowa. Environ Health Persp 114(6):893–897. https://doi.org/10.1289/ehp.8770

Weber RWS, Wakly GE, Pitt D (1999) Histochemical and ultrastructural characterization of vacuoles and spherosomes as components of the lytic system in hyphae of the fungus *Botrytis cinerea*. Histochem J 31(5):293–301. https://doi.org/10.1023/a:1003713901179

Wellburn AR (1994) The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolutions. J Plant Physiol 144(3):307–313. https://doi.org/10.1016/S0176-1617(11)81192-2

Whittake RH (1996) Ecology: theories and applications. Prentice Hall, Upper Saddle River NJ, p 403

Wilson CL, Stiers DL, Smith GG (1970) Fungal lysosomes or spherosomes. Phytopathology 60:216–227. https://doi.org/10.1094/Phyto-60-216

Wolf RE, Friedli C, Lauer B (2009) Comparison of spray droplet size and coverage for nozzles used or agronomic weed control. Ames: ASABE. American Society of Agricultural and Biological Engineers. https://elibrary.asabe.org/abstract.asp?aid=27331

Wolf TM, Harrison SK, Hall FR, Cooper J (2000) Optimizing postemergence herbicide deposition and efficacy through application variables in no-till systems. Weed Sci 48(6):761–768. https://doi.org/10.1614/0043-1745(2000)048[0761:OPHDAE]2.0.CO;2

Wulandari YRE, Triadiati T, Sulistyaningsih Suprayogi A, Rahminiwati M (2021) Salinity stress affects growth and physiology of mulberry (*Morus* sp.). IOP Conf Ser Earth Environ Sci 948:012049. https://doi.org/10.1088/1755-1315/948/1/012049/pdf

Yamauchi N (2015) Postharvest chlorophyll degradation and oxidative stress. In: Kanayama Y, Kochetov A (eds) Abiotic stress Bbology in horticultural plants. pp 101–116 Springer. Japan. Tokyo,

Yang SY, Huang TK, Fuo HF, Chiou TJ (2017) Role of vacuoles in phosphorus storage and remobilization. J Exp Bot 68(12):3045–3055. https://doi.org/10.1093/jxb/erw481