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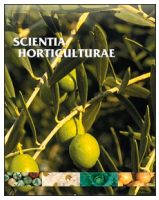

Research Paper

Tracing the origins of phosphonate residues in organic vineyards: A novel analytical approach

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ABSTRACT

In the European Union, organic viticulture faces enormous challenges in controlling grapevine downy mildew since the ban on inorganic phosphonates or phosphonic acid in 2013. However, inorganic phosphonate is still detected in organic wines, although the responsible winegrowers often pledge that they had not sprayed phosphonate-containing products in their vineyards. Among several hypotheses on the origin, the emergence of phosphonate from the soil, e.g., due to preceding applications or from contaminated groundwater, is in discussion. This study investigates whether an analytical differentiation of the origin of phosphonate in the plant or the final product might be feasible by examining leaf and petiole tissue. A total of 908 leaf and petiole samples of various grapevine cultivars were collected from a container vine experiment as well as from four experimental vineyard sites in Germany, on which phosphonate was either sprayed onto the plants as part of crop protection (all experiments), applied to the soil (container experiment only) or present as residue from previous applications (vineyard experiments). Phosphonate concentrations in leaves and petioles depended strongly on whether plants had been sprayed or had taken up phosphonate from the soil. Therefore, an index was created and tested using independent datasets from different geographical locations, based on the concentrations found in leaves and petioles. Index accuracy was at 99.1% correct classifications when distinguishing phosphonate origin from the soil versus that from foliar spraying. Furthermore, phosphonate uptake from the soil was shown to allow considerable phosphonate concentrations in the berries and musts, rendering associations of phosphonate residues in wines with accusations of an actual foliar application highly questionable. In brief, our data and index might provide an approach for identifying the source of phosphonate contamination in the grapevine plant and, if suitable sample material is available, also the related products.

1. Introduction

Organic viticulture relies on a comparably low number of restricted farming practices. These shall not harm or should even promote the health of the soil, the environment, and the plants other than grapevine on or around the vineyard. In particular, the use of synthetic pesticides such as fungicides and herbicides is to be avoided. One of the major challenges in organic viticulture is the control of grapevine downy mildew, a widespread disease that is caused by the oomycete *Plasmopara viticola* and significantly impacts grape yield and quality. Besides applying inorganic salts of copper, which is part of the current state of the art in organic viticulture of the European Union (Regulation (EU) 2018/848), potassium phosphonates had also been widely used until 2013 as plant strengtheners – mainly in organic viticulture – to restrain the ubiquitous pressure of grapevine downy mildew (Bleyer et al., 2020). Inorganic phosphonates are known to foster the grapevine's

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defense mechanism to fungal infections due to several modes of action as described in literature (Gómez-Merino and Trejo-Téllez, 2015; Guest and Grant, 1991; Machinandiarena et al., 2012; Ramezani et al., 2018). However, they also exhibit a direct action on the fungus and their origin is considered non-natural by many organic agricultural associations. Additionally, residues occur in well-quantifiable amounts in both the grapevine organs and in wine when phosphonates have been applied in the vineyard. Consequently, phosphonates were classified as plant protection agents since 2013 (Commission Implementing Regulation (EU) No. 369/2013). At the same time, they were not included in Annex II of Regulation (EU) 2018/848 as plant protection products for organic farming. Therefore, phosphonates should no longer be used in organic agriculture including viticulture from 2013 onwards (Manghi et al., 2021; Trinchera et al., 2020). Nevertheless, due to their positive effects on controlling fungal diseases, some studies suggested that phosphonates would be helpful to keep organic horticulture and especially organic viticulture vital (Bleyer et al., 2020; Dann and McLeod, 2021). Noteworthy, very few alternative substances are available to support reducing or even replacing copper products for combating grapevine downy mildew (Dagostin et al., 2011).

Despite the ban on their use in organic agriculture, however, the presence of very low amounts of phosphonate residues has become a major concern for traders, processors and farmers of organic produce (Del Gómez-Ramos et al., 2020; Gormez et al., 2021). In Germany, the current limits are 0.05 mg/kg for annual or biennial crops (e.g., wheat) and 0.1 mg/kg for perennial crops (e.g., apple and grapevine) according to the Federal Association of Organic Food and Natural Products (Bundesverband Naturkost Naturprodukte e. V., BNN). The strictest limit in the EU is currently set at 0.01 mg/kg for infant food as outlined in Directive (EC) No. 2016/127 (Bundesverband Naturkost Naturwaren, e. V., 2022; Nader et al., 2023).

Upon detecting levels of phosphonate close to the thresholds of legal limits in organic products, harsh discussions arise on the origin of the compound. Besides actively spraying phosphonate for pathogen control in the vineyard, the intake of phosphonate is discussed to potentially result from cross-contamination of neighboring non-organic vineyards, contaminated groundwater or long-term persisting residues in the soil. The use of contaminated materials in the vineyard or cellar, like fertilizers or phosphate salts as fermentation aids, has also been under suspicion as sources of the undesired compound (Nader et al., 2023). The unexpected presence of phosphonate residues in the final product can cause a significant economic impact and threaten the existence of winegrowers, since contaminated organic wines are deemed non-compliant and cannot be sold as organic. Those wines often need to be destroyed as the costs of gathering and re-labeling the bottles are uneconomically high. Methods for differentiating the origin of the phosphonate, i.e., whether the phosphonate occurred due to intentional pesticide spraying or not, are lacking to the best of our knowledge.

In this study, we sought to develop an analytical procedure allowing to distinguish whether a grapevine plant had taken up phosphonate by foliar spraying or by uptake from the soil. For this purpose, we first applied phosphonic acid solutions to vines potted in small containers by either foliar spraying or by directly pouring them onto the soil in the container. An open-field vineyard study was also carried out to corroborate or disprove the findings obtained in container vines. In addition, a set of experiments for a broader validation of our data was conducted on experimental vineyards in four geographically different locations in Germany. We analyzed levels of inorganic phosphonate in different plant parts such as berries, leaves and petioles as well as musts to search for clues allowing the differentiation of the phosphonate origin (foliar spraying vs. soil uptake). Although a differentiation of the origin by analyses of the musts or wines would be the most convenient analytical way, we hypothesized that such product analyses would not allow such a differentiation. Therefore, the aforementioned analyses of leaves and petioles were included, because we speculated to see differences in phosphonate levels depending on the uptake route of phosphonate (foliar spraying vs. soil uptake).

2. Materials and methods

2.1. Brief overview on the different experiments carried out

In the following, the conduction of experiments on container vines (Section 2.2.), in vineyards located in Geisenheim (Section 2.3.) and in vineyards across Germany (Section 2.4.) is described in detail. Data obtained with container vines and the Geisenheim vineyard were utilized to generate the basic idea of differentiating the origin of inorganic phosphonate by analyses of leaves and corresponding petioles. By including further vineyards across Germany, we sought to corroborate or disprove this basic idea. An overview over sample amounts derived from the respective experimental sites is shown in Table 1.

2.2. Experiment with container vines

2.2.1. Grapevine cultivation

One-year-old *Vitis vinifera* L. cv. Riesling shoots from a residue-free trial were grafted on rootstocks of *Vitis riparia* x *Vitis cinerea* cv. Börner by the Department of Grapevine Breeding of Geisenheim University. The grafted shoots were potted into containers (28 × 22.5 × 28 cm^3) with standard soil (ED73®, Einheitserde, Sinntal, Germany) and raised in spring 2020 according to common commercial practice omitting any usage of products containing phosphonic acid. From the following growing season (2021), the vines were consequently irrigated with a single-drop irrigation system (2 L per pot daily) and supplemented with 25 g per pot in mid-May of a long-term stable fertilizer supplying nitrogen (nitrate and ammonium salts), phosphorous (phosphate), potassium (potassium oxide) as well as the micro-nutrients B, Cu, Fe, Mn, Mo, and Zn (Basacote® Plus 6 M, Compo Expert, Münster, Germany) to maintain optimal growth conditions during the vegetation period. The grapevines were trained using a trellis system without fruiting wire. Management practices included two fungicide applications (Sercadis®, BASF SE, Ludwigshafen, Germany) to address powdery mildew and two applications of an insecticide (Confidor®, Bayer CropScience, Leverkusen, Germany), applied from maturation stages 19 to 36 (E-L-stage according to Coombe 1995).

2.2.2. Phosphonate application

With regard to the phosphonate treatments, phosphonic acid (99%, w/w, Sigma-Aldrich, Steinheim, Germany) was diluted with deionized water to 0.4 or 0.54% (w/v) prior to spraying. Approximately 100 mL of the diluted solution were applied to a container vine with a Mesto 3610 high-pressure sprayer (Mesto, Freiberg, Germany) with an initial pressure of 6 bar over ca. 13 s. The 0.4% phosphonic acid solution was sprayed four times at intervals of two weeks, and the more concentrated solution (0.54%) three times at the same intervals, both starting from E-L 15. In order to supply phosphonic acid to the soil to become available for uptake via roots only, an amount of 100 mL of the aforementioned 0.54% (w/v) phosphonic acid solution was poured uniformly onto the soil surface of the pot. All treatments (foliar spraying with 0.4%, 0.54% and soil application with 0.54% phosphonic acid) were performed in biological triplicates each consisting of four neighboring grapevines, i. e., in total 12 grapevines per treatment.

Spraying treatments as described above were stopped after flowering (E-L 26). Except for the above-mentioned fungicide and insecticide applications for ensuring sufficient survival of the young container vines, the complete management of the container vines was in line with the principles of organic farming. The trial was designed for two years.

2.2.3. Sample collection

The container vines were sampled by collecting eight leaves with petioles from each side of the respective row segment, such as north and south, resulting in a total of 16 leaves pooled from four vines

Table 1
Number of leaf and petiole sample pairs used for the development of the analytical procedure, highlighting those used for model generation for the below-described index ($I_{leaf\text{-}petiole}$) and its validation.

	Container vines		Vineyard trial (Geisenheim)			Vineyard trials (Freiburg, Oppenheim, Veitshöchheim)		
	2021	2022	2020	2021	2022	2020	2021	2022
Rootstock	*Vrxc Börner*	*Vrxc* Börner	*Vbxr* 5C Gm	*Vbxr* 5C Gm	*Vbxr* 5C Gm	*Vbxr* SO4/5BB	*Vbxr* SO4/5BB	*Vbxr* SO4/5BB
Cultivar	Riesling	Riesling	Riesling	Riesling	Riesling	M.-T.	M.-T.	M.-T.
Soil application	21	0	28	0	0	12	4	0
Foliar application	87	96	56	56	56	12	14	12
Model generation	x	x	x	x				
Model validation					x	x	x	x

Vrxc: Vitis riparia x *V. cinerea, Vbxr: Vitis berlanderi* x *V. riparia,* 5C Gm: cv. 5C Geisenheim.
M.-T.: cv. Müller-Thurgau.

representing one sample. Samples were taken at continuous intervals (E-L 19, 30, 36, 37) over the entire vegetation period. At each of these points, a total of six samples were taken for the soil treatment, and 12 for each of the above-mentioned two foliar spraying treatments, resulting in targeted 24 and 96 samples, respectively. Due to insufficient growth of the respective young grapevines, only 4 and 5 instead of 6 samples were taken from E-L 19 and 30 (soil application), respectively, while only 9, 8, and 10 instead of 12 were sampled from E-L 19, 30, and 36 (foliar application, Table 1), respectively. No soil application was performed in 2022 and therefore no such samples were collected. Berry sampling was conducted at E-L 37. For this, 80 berries were collected from each bunch of the grape from the same vines. Compared to the vineyard experiment, no must was produced due to the low quantity. Further sample preparation for analyses is described below.

2.3. Vineyard experiment at the Geisenheim site

2.3.1. Grapevine cultivation

The experimental vineyard was located close to Geisenheim, Germany (49° 59′ N, 7° 56′ E) and was planted in 2008 with *Vitis vinifera* L. cv. Riesling grafted on rootstock of *Vitis berlandieri* x *Vitis riparia* cv. 5C Geisenheim with rows orientated east-west. The vineyard was under integrated management until 2019 and then converted to organic practice. Vines were trained using a vertical shoot positioning system (VSP) with one-year old canes pruned to six nodes per m^2. The vineyard was managed organically according to regulation (EU) 2018/848 and after ECOVIN standards. Management practices included the usage of cover crops in every second row, herbicide-free under vine management, gentle soil management, and a moderate, one-sided defoliation after flowering.

The vineyard experiment featured three different plant protection strategies: (i) an untreated control, (ii) a reduced Cu strategy with max. application of 2 kg Cu/ha/a and foliar-sprayed potassium phosphonate, and (iii) a reduced Cu strategy with phosphonate identical to that of group (ii) but including an early leaf removal prior to flowering (E-L 19), i.e. a removal of four basal leaves. Copper treatments were carried out with the commercially available product Funguran® progress (copper hydroxide; Certis Europe, Germany). Furthermore, all plants, including the untreated control were sprayed against powdery mildew with the commercially available sulfur fungicide Stulln® (Belchim Crop Protection Deutschland GmbH, Germany) in 2020 and 2021 and Kumulus® (BASF SE, Germany) in 2022.

2.3.2. Phosphonate application

For application of inorganic phosphonate, the commercial product Veriphos® (Adama Deutschland GmbH, Germany) with 755 g/L (51.7%, w/w) of potassium phosphonate was used until end of bloom. Veriphos® was applied to the vines at stages E-L 17, 19, 22, and 25 using doses of 2.0, 2.0, 2.5, and 2.5 L/ha, respectively. Each dose was diluted with potable water at rates of 270, 270, 360, and 450 L/ha, respectively. This resulted in an application of 1.5 kg/ha of potassium phosphonate for the 2.0 L/ha doses and 1.9 kg/ha for the 2.5 L/ha doses. All foliar spraying applications were performed with an air-assisted tunnel spraying device (TSG-A 1, Lipco, Sasbach, Germany) and according to the good agricultural practice (GAP) (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz – BMELV, 2010) of viticulture management. Plant protection was conducted according to weather conditions and predicted infections provided by the decision support systems (DSS) from Geisenheim University (Berkelmann-Loehnertz et al., 2023).

2.3.3. Sample collection

The experimental vineyard included four field replications for each of the three groups with varying plant protection strategies. Samples of 20 leaves with petioles were randomly collected from each group in each zone, with ten leaves and petioles taken from each side of the vineyard row (for example, north/south) and subsequently pooled. Similarly, at E-L 38, a total of 100 berries were collected from each part of the cluster from the same vines. Additionally, the whole grapes of each variant were harvested, destemmed, and pressed at E-L 38 with a 20 L hydro press (Speidel, Ofterdingen, Germany), according to Otto et al. (2022), to obtain the must samples.

By analogy to the container vine experiment described above, leaf and petiole samples were collected regularly throughout the entire growing period (E-L 25, 32, 36, 38). Eight samples were generated for each treatment at each sampling point, except for E-L 38, where only four samples were collected. This resulted in 28 samples for soil application and 56 for both of the two foliar applications described above. The discrepancies with the numbers in Table 1 arise because some samples lacked residues, preventing index generation. The vineyard trial was identically conducted over three consecutive years. Data from the first two years (2020, 2021) were utilized for the generation of our index model described below. Data from the last year (2022) were included for model validation, alongside with data obtained from samples of the sites described in the following section.

2.4. Vineyard experiments at the sites Freiburg, Oppenheim and Veitshöchheim

Further samples of this study were obtained from three locations, namely Freiburg (47° 58′40.8 N, 7° 50′01.0 E, Baden), Oppenheim (49° 50′43.1 N, 8° 20′54.4 E, Rhineland-Palatinate), and Veitshöchheim (49° 55′24.3 N, 9° 49′25.6 E, Franconia), utilizing an identical sampling protocol, i.e. randomly picking ten leaves with petiole from each side of the row, in total 20 leaves per group and replicate. Additionally, a total of 100 berries were also gathered from each section of the grape clusters from the same vines. In contrast to the Geisenheim site, samples of these sites were only obtained at the grapevines' harvest stage (E-L 38). These trials also adhered to consistent organic management practices in accordance with the EU regulations and according to ECOVIN standards as mentioned above. All three sites utilized a vertical shoot position training system with a flat arch configuration. The Oppenheim and

Veitshöchheim experimental sites featured *Vitis vinifera* L. cv. Müller-Thurgau grapevines grafted onto a *V. berlandieri* x *V. riparia* cv. SO4 (Selection Oppenheim Nr. 4) rootstock, with approximately 20 and 14 years of vine ages, respectively. The Freiburg experimental site comprised cv. Müller-Thurgau grapevines grafted onto a *V. berlandieri* x *V. riparia* cv. 5BB rootstock, planted in 2011.

Within each vineyard trial, two plant protection strategies were set up with four field replicates, i.e. comprising an untreated control and a treatment receiving foliar sprays of potassium phosphonate solution. Treatment applications across all sites were conducted using an air-assisted tunnel sprayer (Schachtner, Ludwigsburg, Germany). Four applications with 2.0, 2.0, 2.5, and 2.5 L/ha Veriphos® diluted with 270, 270, 360, and 450 L/ha potable water were deployed at E-L 17, 19, 22, and 25, respectively. Spraying was performed until E-L 26 (end of flowering). The experimental trials were conducted over a period of three years, from 2020 to 2022.

2.5. Phosphonate analyses

After sampling as described above, leaves and petioles were manually separated, frozen at -20 °C, freeze-dried and subsequently ground (CT 293 Cyclotec, Foss, Hamburg, Germany) to a particle size of ≤ 0.5 mm prior to storage at room temperature (20 °C) until further analyses. Mass loss during freeze-drying was gravimetrically recorded to estimate the dry matter content. Likewise, berries and must were taken and processed following a consistent routine as described by Otto et al. (2022). Sample extraction and IC-ICP-MS analyses were carried out as reported earlier (Otto et al., 2022). The limits of detection (LOD) for leaf, petiole, berries and must were 0.04, 0.03, 0.05 and 0.005 mg/kg and the limits of quantification (LOQ) were 0.12, 0.08, 0.15 and 0.017 mg/kg, respectively. If the residues were below the respective LOD or LOQ in a compartment, the values LOD/2 and LOQ/2 were used for the calculation of the leaf-to-petiole ratio and the $I_{leaf\text{-}petiole}$ index.

2.6. Statistical analyses

Results were expressed as means ± standard deviation unless stated otherwise and expressed as phosphonic acid content on a fresh weight basis (mg/kg) to allow comparison with other studies and facilitate interpretation. The standard deviation of the index ($I_{leaf\text{-}petiole}$) was calculated by the Gaussian law of error propagation from the standard deviations of the respective mean values of individual phosphonate contents and their respective methodological error. Further statistical analyses including the generation of boxplots as well as analyses of variance (ANOVA) and post-hoc Tukey tests were carried out using Microsoft Excel. In addition, a post-hoc Games-Howell test was performed when comparing means originating from different sample sizes.

A total of 908 individual “petiole samples” and “leaf samples” were collected, representing 454 sample pairs of each 16 (container vines) or 20 (field grown grapevines) pooled leaves and petioles as described above and as summarized in Table 1.

3. Results and discussion

3.1. Ambiguity of phosphonate analyses of berries and musts

The major goal of our efforts was to search for an analytical procedure to trace the origin of phosphonate residues in apparently organically produced wines, for which spraying phosphonate on the vineyard is not authorized in the EU. We were interested in differentiating whether such illegitimately present phosphonate residues resulted from active spraying of the plants or from its passive presence in the soil.

As shown in Table 2, our container trials suggested that phosphonate levels in berries from plants that had received phosphonate from the soil (4.6 - 7.0 mg/kg, Table 2) were slightly lower than those from plants that had been sprayed (13.6 - 50.8 mg/kg). In our vineyard trials, the significance of this apparent difference disappeared (soil vs. spray phosphonate: < 0.05 (LOD) - 21.5 vs. 3.2 - 20.3 mg/kg berries and < 0.005 (LOD) - 1.2 vs. 1.2 - 4.6 mg/kg must). According to this data, the sole analyses of phosphonate in wines should not be considered to reliably allow differentiating whether or not a winegrower had sprayed phosphonate. Noteworthy, we have not studied wine directly, but musts and berries. However, if a differentiation is impossible for phosphonate in berries and musts, it should inherently also be impossible for phosphonate in the wine.

The following sections describe an analytical procedure to differentiate the origin of phosphonate in a wine when the corresponding leaf and petiole samples of the respective vineyard are available.

3.2. Phosphonate uptake to leaves and petioles in container and vineyard trials

As shown in Fig. 1, where all leaf and petiole sample pairs across all experiments are displayed (cf. also Table 1), phosphonate levels in both leaves and the corresponding petioles were significantly higher after foliar spraying (gray/black symbols, Fig. 1A) than when taking up phosphonate from the soil (red/orange symbols, Fig. 1A), irrespective of whether the container and vineyard trials were considered. Interestingly, the leaves seemed to have received only very small amounts (on average < 10 mg/kg) when taking up phosphonate from the soil, even when quite large amounts had been supplied to the containers (Fig. 1A and B1). Our results suggest that there was no direct linear relationship between the quantity of phosphonate in the soil and that in the leaves. These data suggest that the sole analyses of the leaves alone might serve as an indicator for the origin of the phosphonate present in the plant. Specifically, we observed levels from 1.3 - 8.5 mg/kg (mean: 5.2 ± 2.0 mg/kg), i.e. not greater than ca. 10 mg/kg leaf, when the phosphonate originated from the soil (Fig. 1A). By contrast, foliar application to container vines led to significantly higher mean concentrations in leaves ranging from 17.3 - 762.1 mg/kg (mean: 139.8 ± 137.6 mg/kg, Fig. 1B1).

While the uptake through the roots consistently led to significantly lower phosphonate levels in the leaves than the uptake through foliar spraying, the levels in the petioles were still lower after soil uptake than after foliar uptake, but overlapped to a slightly greater extent (Fig. 1, a: zoomed view, cf. vertical axis). For instance, in the container vine experiment, petiole levels ranged from 4.4 to 27.0 mg/kg petiole (mean:

Table 2
Average berry and must content of phosphonate after different applications and corresponding average $I_{leaf\text{-}petiole}$ index values (min. - max.).

		Soil application	Foliar spraying
Container trial	**[phos] $_{Berries}$ mg/kg**	6.0 (4.6 - 7.0)	33.4 (13.6 - 50.8)
	$I_{leaf\text{-}petiole}$	2.3 (1.6 - 3.0)	128.4 (9.8 – 1176.9)
Vineyard trials	**[phos] $_{Berries}$ mg/kg**	3.7 (< 0.05 - 21.5)	9.9 (3.2 - 20.3)
	$I_{leaf\text{-}petiole}$	0.4 (0 - 2.6)	365.4 (9.4 – 4336.6)
	[phos] $_{Must}$ mg/kg	0.4 (< 0.005 - 1.2)	2.9 (1.2 - 4.6)
	$I_{leaf\text{-}petiole}$	0.2 (0 - 0.4)	197.3 (9.4 - 583.4)

berries (n = 102), must (n = 26).

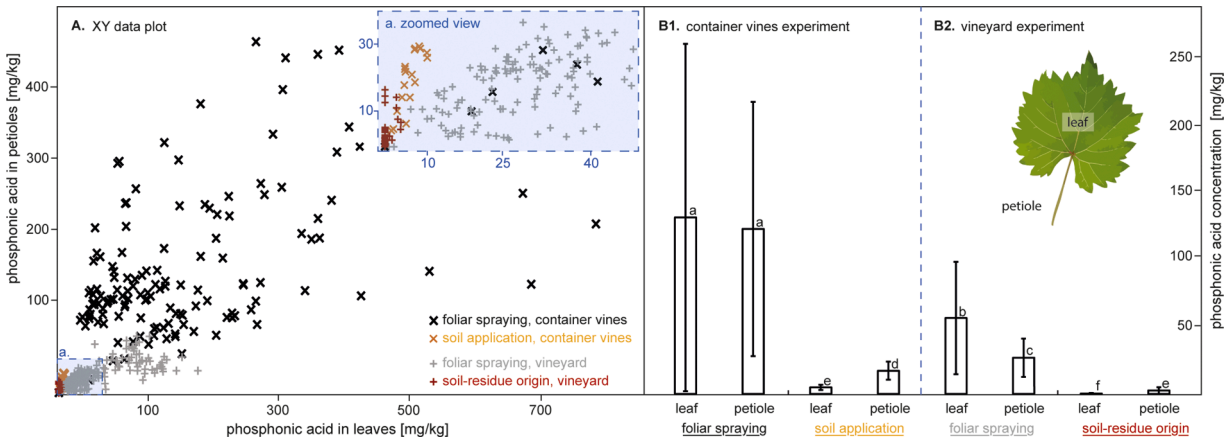

Fig. 1. (A) Concentrations of phosphonic acid in grapevine petioles (vertical axis) and leaves (horizontal axis) across all experiments, i.e. after foliar spraying of container vines (black crosses), after pouring phosphonate to the soil (orange crosses), after foliar spraying in the vineyard (gray pluses), and after phosphonate uptake from residues present in the soil (red pluses).
(B1) Average phosphonic acid concentrations found in petioles and leaves after application of phosphonate by foliar spraying or by pouring on the soil within the container vine trial. (B2) Average phosphonic acid concentrations after foliar spraying or after uptake of residues present in the soil of the vineyard trials across all locations. The bars indicate the standard deviation. Note the inversion of the ratios of concentrations when comparing foliar spraying vs. soil application/soil-residue origin. Different letters indicate significant differences of means at $p < 0.05$.

18.3 ± 7.2 mg/kg) after uptake from soil, being lower than after foliar application ranging from 9.5 to 477.6 mg/kg petiole (mean: 130.7 ± 100.8 mg/kg). As shown in Fig. 1, this difference of phosphonate concentrations in petioles was less pronounced in the vineyard trial. Therein, phosphonate levels in petioles ranged from 0.04 - 76.8 mg/kg (mean: 26.6 ± 16.5 mg/kg) after foliar spraying compared to 0.2 - 13.3 mg/kg (mean: 2.6 ± 2.6 mg/kg) after soil uptake (Fig. 1B2).

The absolute levels in samples of the container vines were higher than those of vineyard samples (Fig. 1A, black crosses and gray pluses). The observed differences in phosphonate exposure between the container and vineyard experiments might have arisen from a combination of factors. The container experiment did not involve air-assisted spraying, which could lead to higher amounts of adhering spray liquid on the leaves and, thus, a more intense load and uptake compared to the standard practice which was applied in the vineyard. Furthermore, the biomass of the container vines was considerably smaller than that of the field-grown vines in terms of canopy size, leading to a higher relative phosphonate exposure per leaf compared to that in the vineyard.

Despite the extensive research on phosphonate uptake and distribution in various crops, there is a lack of knowledge concerning the specific distribution patterns of phosphonates in grapevine tissues, particularly in the context of organic viticulture.

Compared with other studies, our findings on grapevine generally fall within comparable ranges. In potatoes, foliar applications of lower amounts of phosphonate (0.23% solution of mono- and di-potassium salts of phosphonic acid; 10 mL/plant) yielded 166.9 mg/kg in leaves and 76.6 mg/kg in tubers, while applications of higher amounts (0.46%, 10 mL/plant) resulted in 444.2 mg/kg and 265.4 mg/kg, respectively (Borza et al., 2014). Direct root uptake was studied by Ouimette and Coffey (1988) in pepper plants, where they found 342 mg/kg in roots, 186 mg/kg in stems, and 44 mg/kg in leaves when supplying the phosphonate solution directly to the roots. These findings, in particular the ratio between leaf and stem phosphonate levels, are in accordance with our findings of higher levels found in petioles than in leaves after phosphonate uptake through the roots (Fig. 1 B1, B2, soil application, soil residues).

Compared to these findings on concentration and a particular leaf-to-petiole ratio, the pattern was less clear in *Citrus* plants. After supplying phosphonate by a nutrient solution, i.e. leading to uptake through the roots, the concentration was between 0.96 - 2.00 mg/kg in leaves and 2.03 - 3.58 mg/kg in roots, providing evidence for the mobility of phosphonate in *Citrus* (Orbović et al., 2008). Masikane et al. (2020) and McLeod et al. (2018) examined the absorption of phosphonate in avocado tree fruits. For example, Masikane et al. (2020) recorded an intake of 9.3 mg/kg in fruit following a foliar spray and 55.2 mg/kg in fruit after trunk injection. Similarly, in orchard trials, McLeod et al. (2018) observed approximately 60 mg/kg and 40 mg/kg of phosphonate residues in fruit after foliar application and trunk injection, respectively. Additionally, greenhouse investigations on avocados showed that after pot soil drenching with 1 L of phosphonate solution containing 3.2 g fosetyl-Al, phosphonate concentrations of 25 mg/kg in roots, 39 mg/kg in stems, and 99 mg/kg in leaves were detected. When applying a phosphonate solution (6 g of 0.4 g/mL fosetyl-Al) on the stem bark of avocado plants, the concentration increased to 52 mg/kg, 137 mg/kg, and 271 mg/kg in roots, stems and leaves, respectively (El-Hamalawi et al., 1995). Thus, El-Hamalawi et al. (1995) had not observed an inversion of the distribution ratio of phosphonates in the different plant organs as we observed herein in grapevine. This disparity in phosphonate distribution patterns underscores the complexity of this contentious issue in recent horticultural discourse: the distribution of pesticides absorbed from soil and its implications for residue levels in organic farming (Havlin and Schlegel, 2021; Schleiffer and Speiser, 2022).

Our results demonstrated that phosphonate concentrations in grapevine tissues varied depending on route of uptake (Fig. 1 B1, B2). Foliar application led to significantly higher concentrations of phosphonate residues in leaves than upon uptake through the soil. In contrast, uptake through the soil resulted in increased phosphonate levels in petioles, particularly when seen relative to the respective levels in leaves. Therefore, it might be interesting to consider the leaf-to-petiole ratio, alone and in combination with the absolute levels, as described in the following two sections.

3.3. Leaf-to-petiole phosphonate ratio over the vegetation period

The subsequent analysis and discussion are solely based on the container vine experiment and the vineyard trial conducted at the Geisenheim location. As shown in Fig. 1A (zoomed view), the sole usage of phosphonate levels in leaves to trace back the origin of phosphonate might lead to erroneous conclusions, particularly at concentrations around 10 mg/kg per fresh weight. To possibly differentiate the samples better, we examined whether using the ratio of phosphonate concentrations in leaves and petioles would improve the separation as compared to solely looking at phosphonate levels in leaves. This ratio was consistently higher when phosphonate was applied by foliar

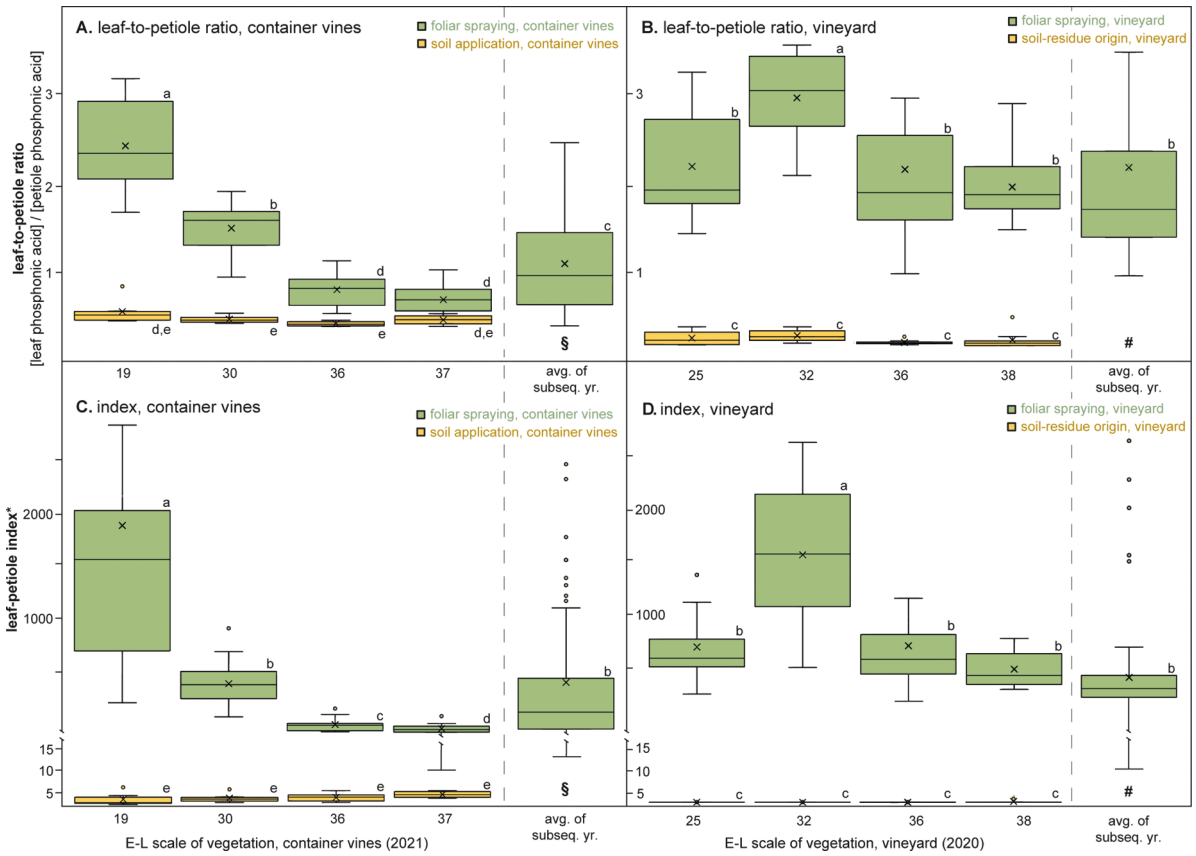

Fig. 2. (A) Ratio of leaf and petiole phosphonate concentrations as found in the container vine experiment over the vegetation period** of 2021 (left) after foliar (green) and soil (yellow) application. Results for the subsequent year (2022) are shown as averages only. (B) The aforementioned ratio is shown for samples from the vineyard trial at Geisenheim over the vegetation period of 2020 and, as average, in 2021. (C) and (D) The developed index ($I_{leaf\text{-}petiole}$) based on identical data as used for (A) and (B) is displayed in C and D, illustrating the improvement of differentiation of treatments. Different letters indicate significances in means at $p < 0.05$. **expressed as E-L code according to Coombe (1995).
§: no samples available for soil application. #: no residue found in the vineyard samples.

spraying as compared to phosphonate uptake through the soil (Fig. 2A and B). In container vine experiments, a decline of the ratios was observed after foliar spraying, i.e. from ca. 2.5 at E-L 19 to ca. 0.6 at E-L 37, (Fig. 2A), potentially caused by a simultaneous decrease in leaf concentration. Possibly, these observations might indicate a distribution of phosphonate into other plant organs after stopping phosphonate spraying (E-L 25). Interestingly, however, such a decline of the ratios was not observed in the vineyard experiment (Fig. 2B), remaining at ca. 0.77 - 3.59 after foliar spraying of phosphonate throughout E-L 19 to 38. The reasons for these deviating behaviors of container and field-grown vines remain unclear, but our observations emphasize the need for open-field trials to be mandatory when having first insights obtained with container vines.

As compared to E-L 25, a significant increase in phosphonate ratios was observed at E-L 32 in 2020 (Fig. 2B). Possibly, this boost in phosphonate concentration was a consequence of the last spraying at E-L 25.

As shown by depicting the average ratios across all samples of the subsequent year in Fig. 2A and 2B, the observed ratios behaved widely consistent in both years. When comparing the ratios in samples of early defoliated vines and those not manually defoliated, no significant difference was observed (data not shown).

When considering phosphonate uptake from the soil, the ratio of its levels in leaves and petioles remained widely constant at 0.2 - 0.7 in the container vine experiment, being slightly lower, i.e. at 0.1 - 0.4 in the vineyard trials, throughout the vegetation period. Compared to the residues absorbed by the leaves, the ratio of phosphonate in leaves to petioles following uptake from the soil remained largely stable throughout the growing season.

Data comparing ratios of leaf and petiole phosphonate concentrations are scarce, but the distribution into other plant tissues or organs has been reported previously. In an earlier study on potatoes, foliar application led to a ratio of approximately 22.7 between phosphonate levels in leaves (500 mg/kg) and those of roots (22 mg/kg) after 48 h (Huang et al., 2018). In addition, Borza et al. (2014) have reported that in fully matured potatoes, a mist application of phosphonate during harvest resulted in a ratio of 2.2 expressed as phosphonate concentration between leaves and tubers. Experiments on eucalyptus have revealed a similar trend. For instance, following spraying or mist application, a ratio of 2.2 or 1.9 of phosphonate in leaves and stems was observed after seven days (Fairbanks et al., 2000). Guo et al. (2021) have shown that soybeans accumulated phosphonate massively in the leaves (ca. 24 g/kg) after treatment, with a leaf-to-stem ratio of 1.3, increasing to approximately 2.7 (ca. 35 mg/kg in leaves) within 48 h.

The ratio of phosphonate levels in leaves to those in stems of avocado was 1.0 with ca. 221 mg/kg in both leaves and stems one week after supplying phosphonate to the soil, then declining to 0.12 with 47 and 382 mg/kg in leaves and stems after eight weeks, respectively. When conducting foliar spraying, the ratio was higher, i.e. at 2.0 with ca. 42 and 21 mg/kg in leaves and stems after one week, however, also declining to 0.25 with 19 and 75 mg/kg after eight weeks, respectively (Ouimette and Coffey, 1989). These findings again clearly highlight the mobility of phosphonate in the plant. In coconut, the phosphonate concentration initially has been observed to increase in the petiole and rachis after trunk injection, and then spread throughout the leaf (Yu et al., 2015). Likewise, after 40 weeks, noticeably lower concentrations were found in the petiole (25 mg/L) compared to the spear leaf (280

mg/L). Nevertheless, it is essential to note that direct comparison of our results to those reported in literature is challenging.

3.4. Development of an index for differentiating phosphonate origin

As a next step, we aimed at combining the above-described concentration ratios with a stronger measure for the absolute concentrations to further optimize distinguishing phosphonate data originating from foliar spraying or soil uptake. All considerations presented in the following are based on data of the container vine experiment in the years 2021 - 2022 and the vineyard trial at Geisenheim site in 2020 - 2021. Although the separation of the aforementioned ratios of phosphonate levels in leaves versus those in petioles was quite clear in the vineyard trials (Fig. 2B), the results of our container vine experiments indicated an unsatisfactory separation, particularly at later ripening stages when coming close to harvest (Fig. 2A). Therefore, we again considered the raw data as illustrated in Fig. 1A and conducted a mathematical transformation to yield a leaf-petiole index ($I_{leaf\text{-}petiole}$), combining (i) the square of the aforementioned ratio as measure for the angle α spanned by the abscissa and the line between origin and the respective data point with (ii) the square root of the sum of the squared single concentrations as a measure for the distance of the data point to the origin as shown in Eq. (1).

$$\mathbf{I_{leaf-petiole}} = \left(\frac{[\mathbf{phos}]_{\mathbf{leaf}}}{[\mathbf{phos}]_{\mathbf{petiole}}}\right)^2 \times \sqrt{[\mathbf{phos}]^2_{\mathbf{leaf}} + [\mathbf{phos}]^2_{\mathbf{petiole}}} \quad (1)$$

Where $I_{leaf\text{-}petiole}$ is the proposed leaf-petiole index, $[phos]_{leaf}$ the concentration of phosphonate found in the leaf sample and $[phos]_{petiole}$ the concentration of inorganic phosphonate in the corresponding petiole sample.

The index $I_{leaf\text{-}petiole}$, as calculated according to Eq. (1), improved the separation of the data, which can be seen by comparing Fig. 2A and C (container experiment) or Fig. 2B and D (vineyard experiment). This improvement was particularly evident for the challenging samples at E-L 36 - 37 in the container vine experiment. For the container vine samples, the average $I_{leaf\text{-}petiole}$ values at different phenological stages showed low values ($I_{leaf\text{-}petiole} < 3.6$) when phosphonate was sourced from the soil, with 1.4 ± 1.2 at stage 19, 1.6 ± 0.9 at stage 30, 1.7 ± 0.8 at stage 36, and 2.2 ± 0.6 at stage 37. Conversely, average $I_{leaf\text{-}petiole}$ values increased significantly when phosphonate was applied through foliar spraying, with $I_{leaf\text{-}petiole}$ means of 1937.9 ± 1865.7 at stage 19, 447.2 ± 209.3 at stage 30, 84.8 ± 61.3 at stage 36, and 49.6 ± 39.6 at stage 37. In the following year, the mean value across all samples where phosphonate had been applied to the leaves was 458.2 ± 682.8. Hence, when comparing $I_{leaf\text{-}petiole}$ indices from container vines (Fig. 2C) and field-grown vines (Fig. 2D) based on their respective application methods at the corresponding time points, a significant difference was observed.

In the Geisenheim vineyard in 2021, the $I_{leaf\text{-}petiole}$ index was consistently higher (9.8 - 3000) after foliar applications than after uptake from the soil (< 3.6). When comparing the early defoliated treatment and the treatment without manual defoliation, no significant difference in the leaf-to-petiole index $I_{leaf\text{-}petiole}$ was observed (data not shown).

According to our results, we propose an acceptance level of the index $I_{leaf\text{-}petiole}$ for identifying samples of grapevines that have taken up phosphonate from the soil ranging from 0 to 5 ($I_{leaf\text{-}petiole}$ value) as based on an error calculation. To determine these limits, the mean $I_{leaf\text{-}petiole}$ value found in samples that had taken up phosphonate from the soil (0.82) was added up with three and six times the standard deviation and the obtained value of 3.97 and 7.12 were rounded up to 5 and 7.5, respectively. At $I_{leaf\text{-}petiole}$ values above 7.5, we postulated that a foliar application appeared very likely. This postulation was challenged by the validation experiment described below.

3.5. Validation of the leaf-to-petiole index by characterization of the foliar and root uptake of phosphonate

To validate and challenge the robustness of the above-mentioned index $I_{leaf\text{-}petiole}$, we applied it to an independent dataset consisting of grapevine samples collected from four different environments, including different varieties (Fig. 3).

To illustrate this, we displayed the indices $I_{leaf\text{-}petiole}$ obtained after analyses of samples from the four study sites in Germany in Fig. 3, being sorted by vineyard location across all vintages (Fig. 3A) and by year across all sites (Fig. 3B).

Across all samples used for validation ($n = 110$), only one sample obtained from vines that had been sprayed was misclassified as a vine exposed to soil phosphonate only by an index $I_{leaf\text{-}petiole}$ of 3.0, which corresponds to an accuracy of 99.1%. In addition, no samples were found within the above-mentioned uncertainty range ($5 < I_{leaf\text{-}petiole} < 7.5$) during validation.

3.6. Considerations on phosphonate levels in berries and musts

In addition to leaf and petiole samples, 128 samples of berries and

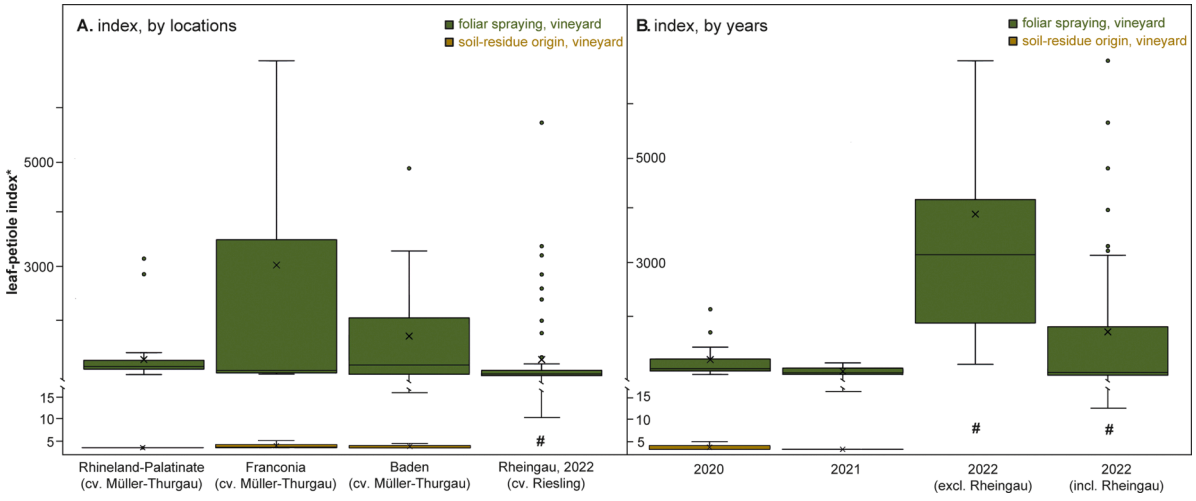

Fig. 3. $I_{leaf\text{-}petiole}$ indices obtained in the validation experiment using independent samples obtained from vineyards of four winegrowing regions in Germany. The data behind both sub-figures are identical, being depicted as categorized by location (A) or year (B).
#: no residue found in samples from vines exposed to soil phosphonate only, i.e. that had not been subjected to foliar spraying.

must harvested at E-L 37 - 38 were analysed (Table 2). In the case of container trials, the mean phosphonate concentration in berries was at astonishingly high values of 6.0 mg/kg fresh berries after soil application, with $I_{leaf\text{-}petiole}$ index values averaging at 2.3. In contrast, foliar spraying resulted in a significantly higher phosphonate concentration of 33.4 mg/kg, and an $I_{leaf\text{-}petiole}$ index value averaging at 128.4. Across all vineyard trials, phosphonate levels averaged 3.7 mg/kg fresh berries (range from 0 to 21.5 mg/kg) even when no phosphonate had been sprayed on the foliage as in the untreated control. Foliar spraying had resulted in significantly high average values (9.9 mg/kg) as expected, but unexpectedly not in a broader overall range (3.2–20.3 mg/kg). Regarding our must samples, which were available in lower quantity, concentrations at around 0.4 mg/kg must were achieved without foliar spraying of the respective grapevines (Table 2).

In brief, no correlation was found between $I_{leaf\text{-}petiole}$ values, representing phosphonate uptake by leaf or root, and concentrations found in the berries and musts. These findings indicate that the sole analysis of phosphonate in berries cannot support the claims that winegrowers sprayed phosphonate as part of their plant protection plan.

By contrast, the $I_{leaf\text{-}petiole}$ index, as deduced from analyses of leaf and petiole samples corresponding to berry samples from the same plants, allowed a clear-cut differentiation of whether the plants had been subjected to foliar spraying or had taken up phosphonate by another route, presumably through the soil.

Our findings align with the results of Nader et al. (2023), who had reported similar phosphonate concentrations in commercial wines and fruits. They found concentrations of 4.3 mg/kg (with a maximum of 50.8 mg/kg) and 0.8 mg/kg (with a maximum of 11.7 mg/kg) in integrated and organic wine samples, respectively. Additionally, they detected 1.0 mg/kg (with a maximum of 120 mg/kg) and 0.03 mg/kg (with a maximum of 0.5 mg/kg) in perennial fruits of conventional and organic origin, respectively, although the commercial origin of their samples does not allow to undoubtedly assign its authentic (organic) origin. Nevertheless, the findings of our controlled experiment confirm to some extent the results of Nader et al. (2023).

4. Conclusion

We conclude that analyses of leaves and petioles of grapevine samples should allow the determination whether a grapevine plant had been sprayed with phosphonate during the current vegetation period or whether the leaves and petioles had taken up phosphonate from the soil or potentially from residues stored somewhere else in the plant, e.g., the stem. We propose an index $I_{leaf\text{-}petiole}$ as particularly useful for the aforementioned differentiation, to be preferred over the sole use of leaf concentrations. Furthermore, we recommend that accusations of spraying phosphonate in the vineyard should not be based solely on phosphonate analyses of berries, musts or wines. We observed a significant amount of berry and must samples with values of phosphonate at ca. 1.0 mg/kg that originated from samples of grapevines that had not been sprayed with phosphonate. In such cases, analyses of leaf and petiole phosphonate can help to clarify whether or not a winegrower has actually sprayed phosphonate. A limitation of the approach is that authentic leaf and petiole samples of the year of a wine's vintage are required and, thus, need to be taken in advance. Also, if no detectable residues are found in both leaves and petioles, our index cannot be used to determine a source of phosphonate intake.

Although we have only shown that berries and musts can reach equivalent concentrations of phosphonate even when no phosphonate has been sprayed, it might be assumed that the transfer factor, i.e. the enrichment or depletion of phosphonate in the subsequent organic and conventional wine production processes, will be somewhat similar. Here, however, further study is required.

CRediT authorship contribution statement

Sören Otto: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft. **Bianca May:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **Beate Berkelmann-Löhnertz:** Resources, Funding acquisition, Project administration, Writing – review & editing. **Randolf Kauer:** Resources, Funding acquisition, Writing – review & editing. **Yvette Wohlfahrt:** Investigation, Writing – review & editing. **Beate Fader:** Investigation, Resources, Writing – review & editing. **Stefan Schumacher:** Investigation, Resources, Writing – review & editing. **Heinrich Hofmann:** Investigation, Resources, Writing – review & editing. **Ralf Schweiggert:** Conceptualization, Validation, Funding acquisition, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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