Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 41(3):375-386. 2014
www.rcia.uc.cl

CROP PROTECTION

DOI: 10.4067/S0718-16202014000300010

RESEARCH PAPER

Effects of wine grape cultivar, application conditions and the winemaking process on the dissipation of six pesticides

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Abstract

C. Alister, M. Araya, J.E. Morandé, C. Volosky, J. Saavedra, A. Cordova, and M. Kogan. 2014. Effects of wine grape cultivar, application conditions and the winemaking process on the dissipation of six pesticides. Cien. Inv. Agr. 41(3):375-386. Pesticide residue in primary products is an important issue for producers and consumers, even though little information is available on the effect of application conditions on residue persistence and the transfer to primary elaborated products. During the 2012 season, field and laboratory studies were conducted to determine the dissipation of lambda-cyhalothrin, buprofezin, pyrimethanil, tebuconazole, imidacloprid and acetamiprid in Sauvignon blanc and Pinot Noir cultivars and their residue dynamics during the winemaking process. Half-life values (DT_{50}) for each pesticide applied alone and as a tank mix of all pesticides were similar and had averages of 6.4, 14.0, 19.7, 26.0, 14.5 and 13.4 days for lambda-cyhalothrin, buprofezin, pyrimethanil, tebuconazole, imidacloprid and acetamiprid, respectively. The grape cultivar did not affect pesticide DT_{50}. All pesticides were transferred from the raw material (grape) to red and white wines except lambda-cyhalothrin. The transfer factors of buprofezin, tebuconazole pyrimethanil, imidacloprid and acetamiprid ranged from 3 to 23% in red wine and 9 to 30% in white wine. Alcoholic fermentation, pressing (through pomace) and malolactic fermentation were the steps in which the greatest residue losses occurred in red wine, whereas pressing (through the grape and stem), alcoholic fermentation and clarification with bentonite had the greatest residue loss in white wine. In both cases, bottled wine showed substantial residue reduction after ten months.

Key words: Pre-harvest interval, residues, transfer factor, wine

Introduction

The presence of a residue in wine leads to rejection by the consumers, even with concentrations below the maximum residue limits (MRLs). According to studies performed in Italy, approximately 30% of foods showed residues below MRLs (Pasarella *et al*., 2009). The main products that provide residues to a person's diet were fruits and wine, comprising 77 and 15% of intake residues, respectively (Lorenzini, 2007).

Received April 24, 2014. Accepted September 5, 2014.
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For this reason, all agricultural productive chains, including wine production, require scientific information related to residue dissipation to avoid food contamination.

The main factors that regulate fruit pesticide persistence include plant species, cultivar, pesticide formulation, application methods, climatic conditions, and pesticide physico-chemical properties and industrial processes (Cabras *et al*., 1997; Cabras and Angioni, 2000; Mandal *et al*., 2010). However, information related to the interaction between these different factors and how each factor affects field dissipation, and therefore the residue level in the processing product, is highly variable (Banerjee *et al*., 2006; Pasarella *et al*., 2009; Gonzalez-Rodriguez *et al*., 2009; Liu *et al*., 2012).

Because of pest resistance in the field, pesticides are normally applied as tank mixtures. However, the effects of the above mentioned factors in pesticide dissipation are not well understood. For these reasons, the aim of this study was to determine the field dissipation of pesticides regularly used on wine grape cultivars (Sauvignon Blanc and Pinot Noir) and to identify the steps of the winemaking process that may affect pesticide residue persistence.

Materials and methods

Pesticide field dissipation studies

The present study was conducted from February to April 2012 in a vineyard located at Casablanca Valley, Valparaiso region, Chile (Latitude 33°17' S and Longitude 71°24' W). Wine grape cultivars corresponded to Sauvignon Blanc and Pinot Noir. Two 40-m rows of each cultivar received an individual application of each of the formulated pesticides (Table 1); additionally, two rows without pesticide application (untreated grapes) were left as a control experiment, and in the case of the Sauvignon Blanc cultivar, two rows were treated by a tank mixture containing all six pesticides. Each treatment was separated by four rows that acted as a "buffer area" to avoid contamination by drift. The formulated pesticides, which included lambda-cyhalothrin, buprofezin, pyrimethanil, tebuconazole, imidacloprid and acetamiprid, were applied using a spray gun (GunJet®) equipped with an Albuz ATR 80 nozzle at 6 bar, achieving an application volume and the rates shown in Table 1.

Grape berry samples were collected in each experimental plot at 0 (after application dryness), 2, 9, 20, 30, 40 and 50 days after application (DAA) following a random sampling in each

Table 1. Pesticides applied to Sauvignon Blanc and Pinot Noir grapes.

Pesticide[1]		Sauvignon Blanc		Pinot Noir	
Active ingredient	Formulation	Application volume (L ha^{-1})	Formulated pesticide rate (mL ha^{-1})	Application volume (L ha^{-1})	Formulated pesticide rate (mL h^{a-1})
Lambda-cyhalothrin	Concentrated emulsion (EC)	1,600.9	249.4	1,162.9	181.1
Buprofezin	Wettable powder (WP)	1,726.4	1,726.4	1,226.0	1,226.0
Pyrimethanil	Concentrated suspension (SC)	1,792.8	2,686.2	1,337.7	2,006.6
Tebuconazole	Water emulsion (EW)	1,635.8	3,052.4	1,444.4	2,695.2
Imidacloprid	Wettable powder (WP)	1,745.5	523.6	1,529.7	458.9
Acetamiprid	Wettable powder (WP)	1,721.7	258.2	1,463.7	219.5

[1]A tank mixture of all them was applied at the same commercial pesticide rate and at an application volume of 1,655.6 L ha^{-1}.

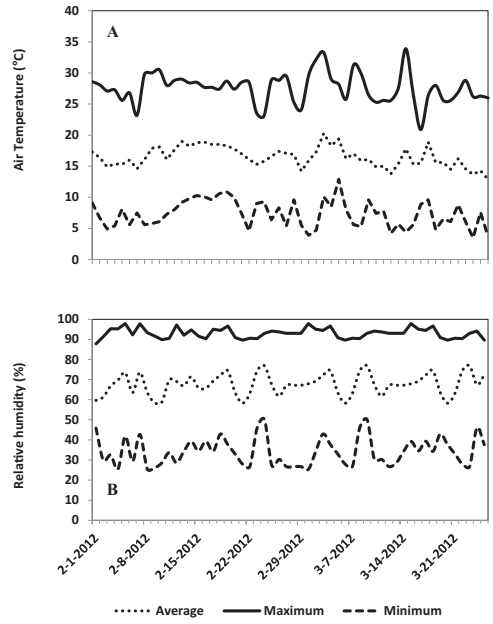

Figure 1. Principal climatic conditions during the study period. A) Maximum, minimum and average air temperature and B) Maximum, minimum and average relative humidity. (Application date: January 1st).

replication (three for each treatment and cultivar). Samples of ±600 g were kept in a plastic bag at 4±1 °C until they were transported to the laboratory, where they were maintained at -18 °C until residue analysis. The climatic conditions are shown in the Figure 1.

Vinification studies

Seventy-two hours after the Pinot noir and Sauvignon blanc grape cultivars were sprayed with the pesticides treatments, 75 kg of each cultivar were harvested and placed in a micro-cellar at the Universidad Viña del Mar. The Pinot noir grapes were crushed, de-stemmed, and transferred to a 200 L stainless steel tank, and 2 mg L^{-1} of SO_2 (sodium metabisulfite) was added. At the end of alcoholic fermentation, the must was separated into free-run wine, press wine and pomace. The free-run and press wines obtained were mixed and transferred to a 50 L stainless steel tank to complete the malolactic fermentation. Afterwards, 2 mg L^{-1} of SO_2 (sodium metabisulfite) were added, and the wine filtered through a 0.2 µm fiberglass filter and bottled.

In the case of the Sauvignon blanc, the grape bunches were pressed, and the obtained juice was transferred to a 50 L stainless steel tank. At the end of the alcoholic fermentation, the wine was clarified using bentonite (0.45 g L^{-1}) and 8 mg L^{-1} of SO_2 (sodium metabisulfite), stabilized at 4 °C for 5 days, filtered through a 0.2 mm fiber glass filter and bottled.

Each vinification process (red and white) was performed in duplicate, and samples for residue analysis were taken in each of the vinification steps, including a final sample after 10 months from the storage bottles, which were stored in darkness at 20 °C and 80% relative humidity.

Pesticide extraction and analysis

In the cases of grape berries, stems and pomace, ±600 g samples were homogenized using a Grindomix Knife Mill, and sub samples of 10 g were taken for analysis. In the case of wine samples, 10 g were taken. All samples were placed in 50 mL plastic tubes to which 20 mL of acetonitrile/methanol was added. After agitation, the plastic tubes were placed into an Ultrasonic bath for 10 min, 4 g $MgSO_4$ and 1 g NaCl were added, and the tubes were shaken. Finally, all samples were centrifuged at 4,500 rpm for 5 min, and an aliquot of 15 mL was taken from each centrifuged sample and concentrated to dryness in a rotary evaporator, re-suspended in 2 mL ethyl acetate, transferred to a 1.5 mL glass vial, and analyzed using high-pressure liquid chromatography (Hitachi LaChrom Elite Model L-2300) with a diode array detector (Hitachi LaChrom Elite Model L-2450) or gas chromatography (Shimadzu Model GC-2010) with a mass detector (Shimadzu GCMS-QP 2010 Plus), depending on the analite.

Lambda-cyhalothrin, buprofezin, pyrimethanil and tebuconazole were quantified through GC-

MS equipped with a RTX® 5-MS 30 m × 0.25 mm × 0.25 mm column. The gas carrier was He, at a flow rate of 1 mL min^{-1}, and the injector temperature was 250 °C. The samples (2 µL) were injected into the autosampler in a splitless mode. The oven temperature was 70 °C for 1 min, increased to 150 °C at 25 °C min^{-1}, followed by an increase to 200 °C at 3 °C min^{-1}, and finally raised to 280°C at 8 °C min^{-1}. Ion Mass (m/z) pesticide quantifications were 181 for lambda-cyhalothrin (time retention: 29.21 min); 105 for buprofezin (time retention: 24.06 min); 198 for pyrimethanil (time retention: 13.845 min); and 125 for tebuconazole (time retention: 23.183 min). The detection limits were 0.0029 mg kg^{-1} for lambda-cyhalothrin, 0.0242 mg kg^{-1} for buprofezin, 0.0114 mg kg^{-1} for pyrimethanil, and 0.0086 mg kg^{-1} for tebuconazole, and the recoveries were 91.0, 108.0, 88.0 and 102.1%, respectively.

Imidacloprid and acetamiprid were quantified through HLPC-DAD. The HPLC unit was equipped with a Waters® Symmetyl Shield RP-8 5 µm 3.9-150 mm column and Chromolith RP-18e 5-4.6 mm pre-column. The liquid phase used was water-acetonitrile at a flow rate of 1 mL min^{-1} with a gradient from water-acetonitrile (95/5 v/v) for 1 min, then to 85/15 (v/v) for 5 min, to 60/40 (v/v) for 2 min, to 20/80 (v/v) for 9 min, and kept at 95/5 (v/v) for 14 min. The column temperature was 35 ºC, and the injection volume was 10 µL. The detector (Hitachi model Elite LaChrom L-2450) settings were 270 nm for imidacloprid (retention time: 12.29 min) and 247 nm for acetamiprid (retention time: 21.487 min). The recoveries of the spiked samples were 99.3 and 100.8% for imidacloprid and acetamiprid, and the detection limits were 0.0112 mg kg^{-1} and 0.0199 mg kg^{-1}, respectively.

Data analysis

Field grape berry pesticide dissipation results were fitted to a first-order kinetic model [1] using nonlinear regression analysis, defined by the following equations:

$$C = Co * \exp(-k * t) \qquad [1]$$

$$DT_{50} = Ln2 / k \qquad [2]$$

$$DT_{90} = Ln9 / k \qquad [3]$$

where C (mg kg^{-1}) is the grape pesticide concentration at time t (days), Co (mg kg^{-1}) is the initial pesticide concentration, and k (days-1) is a first-order dissipation rate that determines the slope of the curve. The model prediction capacity was calculated using a Mean Absolute Error (MAE). The 50% dissipation time (*DT50*) and 90% dissipation time (*DT90*) were estimated using equations 2 and 3.

Residue results from the winemaking process were explored with Principal Component Analysis (PCA) and modeled by Partial Least Square Regression (PLS) and Ridge Regression. The data were sorted into a two-dimensional matrix array, with one dimension as the residue transferred rate and the other dimension as the physico-chemical pesticide properties.

Results and discussion

Pesticide grape berry dissipation

Pesticide field dissipation was well described by a first-order exponential model for all study conditions (individual applications, tank mixture of all pesticides and vine grape varieties) (Table 2). The use of an n>1-order exponential model did not show a significant improvement (data not shown), which is consistent with other field studies (e.g., Pasarella *et al*., 2009; Liang *et al*., 2012). Several authors have reported a higher dissipation rate in the field during the first week after application, followed by a slow dissipation rate during the second or third weeks for table and wine grapes (Cabras *et al*., 2001; Mandal *et al*., 2010; Liu *et al*., 2012). However, in this study, dissipation rates were stable during the entire study period (approximately six weeks). Only

Table 2. First-order dissipation model parameters for six pesticides applied to Sauvignon Blanc and Pinot Noir wine grape berries. Parentheses denote 95% confidence intervals.

Pesticides	Application Condition[1]	Parameters[1]				Residues at 49 DAA	MAE[4]	R^2
		Co	*K*	DT_{50}[3]	DT_{90}[3]	mg kg^{-1}		
Lambda-cyhalothrin	Individually S.B	0.022 (0.019-0.024)	0.048 (0.044-0.053)	14.4 ± 1.4	45.5 ± 4.4	0.003 ± 0.003	0.264	0.93
	Individually P.N	0.024 (0.021-0.027)	0.045 (0.039-0.051)	15.3 ± 2.0	48.6 ± 6.5	0.004 ± 0.003	0.334	0.88
	Mixed S.B	0.020 (0.017-0.022)	0.038 (0.028-0.052)	18.5 ± 3.3	58.6 ± 10.5	0.003 ± 0.002	0.239	0.93
Buprofezin	Individually S.B	3.711 (3.321-4.101)	0.064 (0.053-0.069)	10.8 ± 0.9	34.3 ± 2.9	0.259 ± 0.155	0.302	0.95
	Individually P.N	3.999 (3.626 -4.372)	0.073 (0.056-0.090)	9.8 ± 1.4	31.2 ± 4.3	0.372 ± 0.167	0.404	0.94
	Mixed S.B	2.970 (2.601-3.339)	0.053 (0.047-0.058)	13.2 ± 1.3	41.7 ± 3.9	0.259 ± 0.121	0.316	0.92
Pyrimethanil	Individually S.B	3.018 (2.642-3.393)	0.035 (0.031-0.038)	19.8 ± 1.7	62.7 ± 5.6	0.535 ± 0.077	0.192	0.94
	Individually P.N	2.696 (2.362-3.030)	0.033 (0.030-0.037)	20.7 ± 2.2	65.6 ± 6.9	0.506 ± 0.078	0.231	0.91
	Mixed S.B	3.111 (2.760-3.462)	0.035 (0.032-0.038)	19.6 ± 1.8	62.2 ± 5.3	0.505 ± 0.114	0.196	0.93
Tebuconazole	Individually S.B	3.363 (2.994-3.731)	0.030 (0.022-0.038)	24.3 ± 3.8	77.2 ± 12.2	0.913 ± 0.212	0.137	0.83
	Individually P.N	3.317 (2.931 -3.702)	0.026 (0.024-0.030)	25.2 ± 2.5	79.9 ± 7.8	0.811 ± 0.267	0.168	0.92
	Mixed S.B	3.446 (3.181-3.711)	0.026 (0.023-0.027)	26.9 ± 2.1	85.5 ± 6.7	0.902 ± 0.123	0.117	0.95
Imidacloprid	Individually S.B	0.600 (0.564-0.635)	0.054 (0.047-0.062)	13.0 ± 1.1	41.2 ± 3.3	0.017 ± 0.031	0.218	0.97
	Individually P.N	0.742 (0.677-0.807)	0.050 (0.040-0.060)	14.4 ± 1.7	45.5 ± 5.4	0.057 ± 0.051	0.076	0.93
	Mixed S.B	0.748 (0.659-0.836)	0.043 (0.038-0.047)	16.2 ± 1.6	51.2 ± 5.1	0.090 ± 0.045	0.248	0.92
Acetamiprid	Individually S.B	0.731 (0.657-0.804)	0.060 (0.045-0.074)	12.1 ± 1.7	38.3 ± 5.5	0.035 ± 0.061	0.073	0.92
	Individually P.N	0.673 (0.612-0.733)	0.046 (0.037-0.056)	15.4 ± 1.9	48.8 ± 6.0	0.089 ± 0.030	0.184	0.92
	Mixed S.B	0.675 (0.615-0.735)	0.047 (0.038-0.057)	15.0 ± 1.8	47.7 ± 5.8	0.069 ± 0.062	0.061	0.92

[1]Individually S.B= Each pesticide applied individually to Sauvignon Blanc wine grapes; Individually P.N= Each pesticide applied individually to Pinot Noir wine grapes; Mixed S.B= Six pesticides applied as a tank mixture of all pesticides to Sauvignon Blanc wine grapes.
[2]Co= Initial fruit pesticide concentration (mg kg^{-1}); k = First-order dissipation constant ($days^{-1}$); DT50 and DT90 = 50% and 90% dissipation time (days).
[3]Values are means of three replications ± SE.
[4]Medium average error.

in the cases of buprofezin and pyrimethanil different dissipation rates were observed, although the higher dissipation rate occurred during the first three weeks.

In general, all pesticides showed detectable residues until the end of the study period (50 DAA). However, considering the MRLs for wine grapes, all pesticides satisfied the most restricted worldwide MRLs, established as follows: Lambda-cyhalothrin = 0.01 mg kg^{-1}, buprofezin = 0.3 mg kg^{-1}, pyrimethanil = 2.0 mg kg^{-1}, tebuconazole = 1.0 mg kg^{-1}, imidacloprid = 0.1 mg kg^{-1} and acetamiprid = 0.05 mg kg^{-1}. In this study, those MRLs were reached at 12, 25, 9, 35, 29 and 49 days, respectively.

Thorbek and Hyder (2006) considered that 50% of pesticide dissipation is related to physico-chemical properties. However, other factors, such as weather conditions, variety, application techniques and fruit growth stage, could affect pesticide dissipation curves (Benerjee *et al*., 2006). The results of this study did not show any significant difference in pesticide dissipation whether the pesticides were applied individually or in a tank mixture or between different wine grape cultivars (Table 2).

Lamda-cyhalothrin DT_{50} values were similar to values reported for apple foliage and fruits (Jun *et al*., 2008) but higher than those reported for wine grapes (*Vitis vinifera*) (Benerjee *et al*., 2006). In the case of wine grapes, the DT_{50} was between 4.7 and 7.0 days for almost 50% of the DT_{50} values obtained in our study. This difference could be explained because applications were made at the end of the fruit growth period,

in comparison to the mentioned study (Benerjee *et al*., 2006), in which pesticides were applied at the initial stage of grape berry growth. Similar to lambda-cyhalothrin, buprofezin showed higher dissipation rates than rates reported for other species, such as clementines (*Citrus reticulate* var. *clementina*) (Cabras *et al*., 2001) and wine grapes (Oulkar *et al*., 2009).

Gabriolotto *et al*. (2009) found a range between 0.24 and 0.47 mg kg^{-1} of pyrimethanil residues in wine grape berries at 65 DAA, which is consistent with the results of this study (Table 2). Moreover, these authors found the same dissipation rate for pyrimethanil and five other pesticides in Moscatel and Barbera wine grape cultivars. These results are contradictory to those of Angioni *et al*. (2006), who reported a DT_{50} of 12 days for table grapes, with an initial deposit of 2.48 ± 0.9 mg kg^{-1}, in concordance with results presented here. However, Cabras *et al*. (2001) reported a DT_{50} of 57 days for the same pesticide in the same species.

In the case of tebuconazole, reported dissipation rates have been variable. Jyot *et al*. (2010) determined a DT_{50} value of less than 5 days and no detectable residues at 34 DAA when a mixture of trifloxystrobin + tebuconazole was applied to grape berries. In contrast, Mohapatra *et al*. (2010) found tebuconazole residues in grape berries at 30 DAA and a DT_{50} of approximately 20 days after the same mixture was applied. In this study. Tebuconazole DT_{50} varied between 16.6 and 31.5 days, depending on the types of grape cultivars and pesticides used (Table 2). The residues were approximately 0.876 ± 0.056 mg kg^{-1} at 49 DAA. This difference between our results and the few reported in the literature could be explained by a dilution effect resulting from fruit growth and possible losses from sunlight photo-degradation and co-distillation (Pasarella *et al*., 2009).

Acetamiprid and imidacloprid also showed variable results in comparison to the literature. Imidacloprid DT_{50} varied from 13.0 to 14.4 in Sauvignon Blanc and Pinot Noir, respectively (Table 2), with residues of 0.055 ± 0.037 mg kg^{-1} at 49 DAA. A similar dissipation rate was observed by Mohapatra *et al*. (2011) in wine grape berries, reporting a DT_{50} of 16.6 days and residues of 0.074 mg kg^{-1} at 50 DAA. However, Arora *et al*. (2009) determined residues of 0.14 mg kg^{-1} at 15 DAA. Acetamiprid dissipation in the present study showed a DT_{50} 12 days longer than that reported by Arora *et al*. (2009) and Gupta *et al*. (2005), and residues were detectable up to 49 DAA (Table 3). These substantial differences could be related to sunlight (UV-light) and temperature conditions, factors important for acetamiprid field dissipation (Gupta *et al*., 2008).

Table 3. Pesticide transfer rate (%) resulting from both vinification process evaluated before bottling and after bottle storage (10 months in darkness at 20±2 °C). Values correspond to the average of two replications ± standard deviation.

Pesticide	Transfer rate (%)			
	Bottled wine		After storage in bottle	
	White wine	Red wine	White wine	Red wine
Lambda-cyhalothrin	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
Buprofezin	10.7 (±3.2)	12.0 (±0.2)	2.2 (±0.9)	1.8 (±0.2)
Pyrimethanil	10.4 (±2.3)	8.8 (±1.6)	6,3 (±1.1)	4.0 (±0.7)
Tebuconazole	9.0 (±2.5)	3.3 (±0.1)	2.5 (±1.2)	1.5 (±0.1)
Imidacloprid	13.9 (±3.1)	10.4 (±2.0)	1.2 (±1.0)	0.7 (±2.0)
Acetamiprid	30.4 (±9.8)	23.2 (±5.1)	4.5 (±3.3)	8.6 (±5.1)

Table 4. Ridge regression to pesticide residue transfer (%) from grape to wine.

Wine making process	Ridge Regression Model[1]	R^2
White	Transfer % = 0.039 – 0.0022**LogKow* + 9.298E-5**S* + 1.758E-3* WTD_{50}	0.87 (P≤0.0001)
Red	Transfer % = 0.746 – 0.00154**LogKow* + 7.969E-5**S* + 0.252E-3* WTD_{50}	0.80 (P≤0.0001)

[1]S=Solubility at 20°C; WTD_{50}= Water half-life.

Pesticide residues during the vinification process

The transfer percentage from grape berries to wine, for all pesticides studied, was between 0 and 43.9% in white wine and 0 and 23% in red wine. However, the transfer rate considering bottled wine after the storage period (ten months at darkness at 20±2 °C) decreased the maximum transfer percentages to 10.4 and 8.6% for white and red wine, respectively (Table 3).

Data from the literature on pesticides transfer from grape berries to wine are variable and dependent on the vinification process and physico-chemical pesticide properties, particularly the lipophilicity (LogKow) and solubility (Cabras *et al*., 1997; Navarro *et al*., 1999; Cabras and Angioni, 2000; Agnioni *et al*., 2003; Gonzalez-Rodriguez *et al*., 2009).

The PLS and PCA analyses determined that the pesticide residues transfer in both winemaking processes (red and white) depended on the LogKow, solubility and water DT_{50}. The Ridge Regression showed coefficients of 0.87 (P≤0.001) and 0.80 (P≤0.001) for red and white wine, respectively (Table 4).

In general, less residue removal was found in white grape berry vinification process compared to the red vinification process (Table 3; Figure 2 and 3). Angioni *et al*. (2011) found more removal of iprovalicarb, indoxacard and boscalid in red wine vinification than in the white wine process. The higher residue removal observed in red wine vinification is in part due to the malolactic fermentation, which reduced pesticide residue from 44 to 76%, particularly in the cases of tebuconazole and imidacloprid, respectively (Figure 2). This fact is in concordance with several studies that have shown the importance of the fermentation process in residue losses (Navarro *et al*., 1999; Cabras and Angioni, 2000; Fernandez *et al*., 2005; Gonzalez-Rodriguez *et al*., 2009). However, some of these authors did not found effect of malolactic fermentation on quinoxyfen, mepanipyrim, carbaryl, carbendazim, chlorothalonil, fenarimol, metalaxyl, oxadixyl, procymidone, triadimenol or tebuconazol losses (Cabras *et al*., 1999; Gonzalez-Rodriguez *et al*., 2009).

Pomace represented the other critical step in which residues were reduced in both white and red wine vinification. Bentonite clarification was additionally important in reducing residues in white wine vinification. The importance of pomace in removing residues was directly related to the pesticide LogKow. Thus, pomace explained over 50% of tebuconazole, pyrimethanil and buprofezin removal and 100% of Lambda-cyhalothrin removal. However, for the most hydrophilic compounds, imidacloprid and acetamiprid, this step did not result in a significant effect (Figures 2 and 3). Gonzalez-Rodriguez *et al*. (2009) found that over 88% of tebuconazole was detected in pomace and only 12% at the end of the alcoholic fermentation in red wine. The same results were reported for cyprodinil, fludioxonil, pyrimethanil and quinoxyfen (Fernandez *et al*., 2005). Other researchers found no residues of triazole fungicides at the end of the alcoholic fermentation in red wine (Cabras and Angioni, 2000).

Clarification with bentonite had an important effect only for buprofezin. Some researchers have observed a strong effect of the clarification

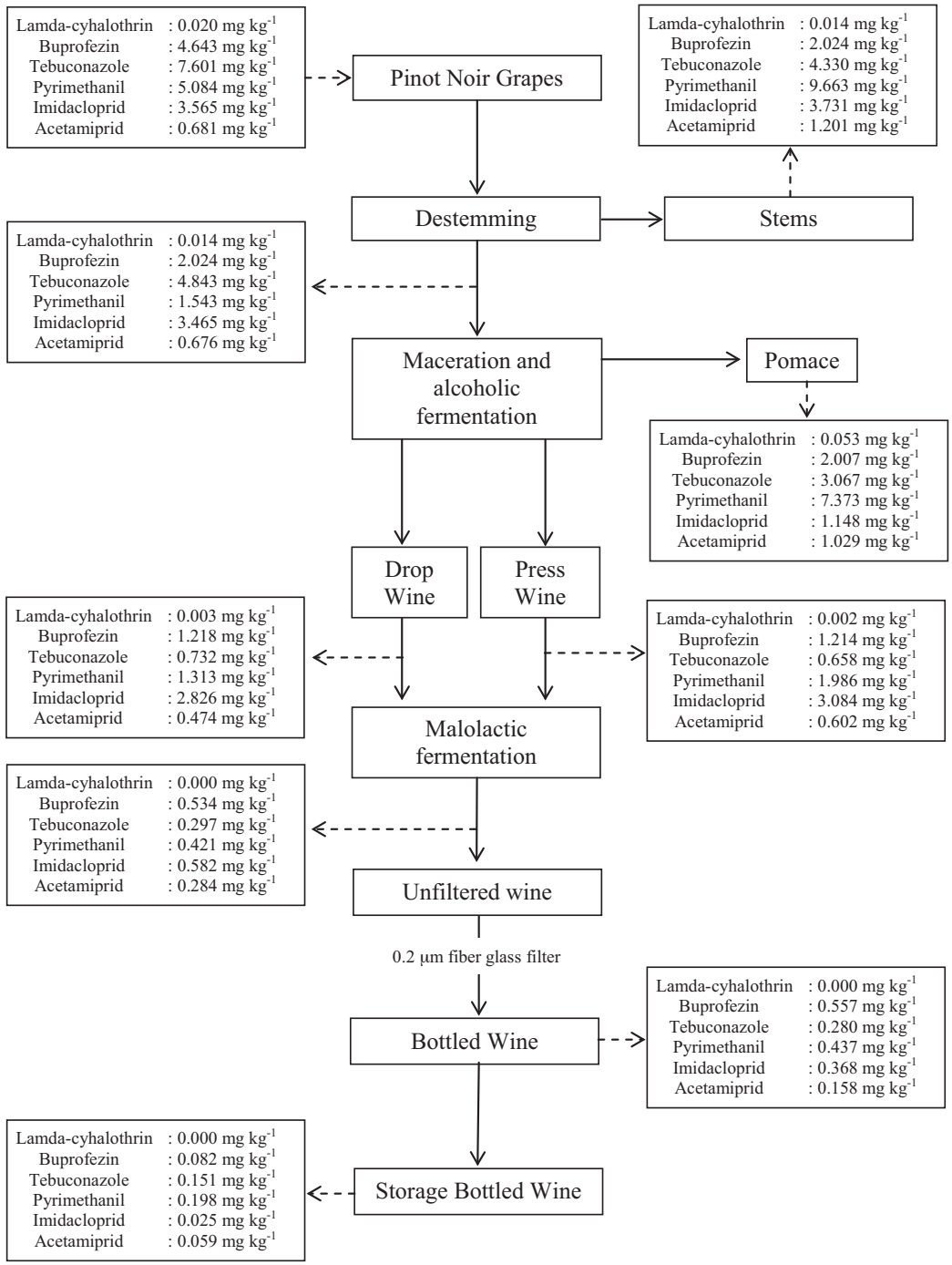

Figure 2. Red wine vinification flow diagram, sample steps and pesticide concentration in each critical stage. Values correspond to the average of two replications.

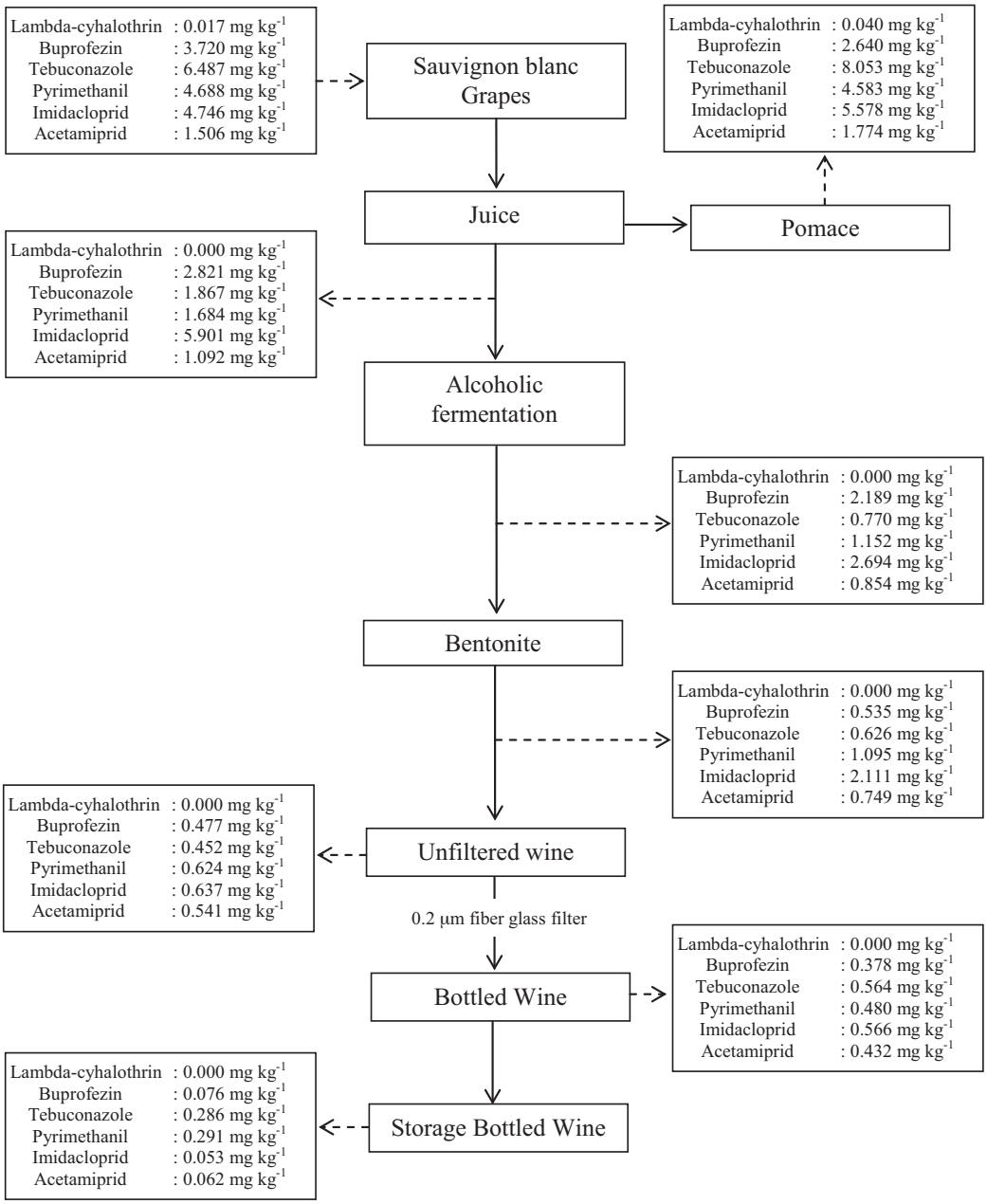

Figure 3. White wine vinification flow diagram, sample steps and pesticide concentration in each critical stage. Values correspond to the average of two replications.

process in the removal of pesticide residues, but these results are variable depending on the type of pesticide and the clearing substance (Navarro *et al*., 1999; Cabras and Angioni, 2000; Fernandez *et al*., 2005; Gonzalez-Rodriguez *et al*., 2009; Angioni *et al*., 2011). According to Cabras *et al*. (1997), bentonite was able to remove only cyprodinil, but not fludioxonil, pyrimethanil and tebuconazole, and charcoal was the only effective clearing substance, as it removed over 90% of the pesticides. Oliva *et al*. (2007) did not find a significant effect of bentonite plus gelatin as removal substances for famoxadone, fluquinconazole and trifloxystrobin. Similarly, Likas and Tsiropoulos (2011) were able to remove tebufenozide with charcoal without using bentonite, gelatin, PVPP or potassium caseinate.

Filtration through a fiber glass filter did not show any significant effect on pesticide residue removal in both white and red vinification processes. Contrary results can be found in the literature regardless of filtration and clarification practices used, though results depend on the types of pesticides and matrix (Navarro *et al*., 1999; Fernandez *et al*., 2005; Oliva *et al*., 2007).

Wine bottle storage was also critical in residue dissipation. All pesticides were reduced over 70% in both red and white wine (Table 3). Limited information exists about the effect of wine storage on pesticide persistence. Navarro *et al*. (1999) found reductions of fenarimol, vinclozolin, penconazole and metalaxyl residues of approximately 37, 31, 26 and 14%, respectively, after 180 days of storage. Stavropoulus *et al*. (2001) found rapid degradation of methidation and pyrazophos in red and white wine during bottled storage, with a DT_{50} of less than 34 days.

According to the results, the pesticide dissipation curves were the same for white and red wine grape berry cultivars and are well described by a first-order dissipation model. Additionally, dissipation rates were consistent between individual or mixed pesticide treatment and both types of cultivars.

The principal steps in which pesticide residues could be reduced are alcoholic and malolactic fermentations and bottled wine storage. However, residue removal by pomace and/or clarification (bentonite) could be variable, depending on the type of pesticide.

Finally, further understanding of pesticide transfer factors to wine, removal of residues in vinification processes and bottle storage, and pre-harvest intervals for wine grapes are measures that could successfully assure that future wines contain no pesticide residues.

Acknowledgements

The authors wish to thank FONDECYT (Chilean Fund for Science and Technology) for funding Project Number 1120925 and ANASAC Chile S.A. and SIDAL Ltda. for their support.

Resumen

C. Alister, M. Araya, J.E. Morandé, C. Volosky, J. Saavedra, A. Cordova y M. Kogan. 2014. Efecto del cultivar, condiciones de aplicación y proceso de vinificación en la disipación de seis plaguicidas en uva vinífera. Cien. Inv. Agr. 41(3):375-386. La presencia de residuos de plaguicidas en productos agrícolas primarios es un tema muy importante para los productores y consumidores, sin embargo la información existente respecto al efecto de las condiciones de aplicación sobre la persistencia de los residuos y su potencial traspaso a productos elaborados es limitada. Durante la temporada 2012, se desarrollaron estudios de campo y laboratorio destinados a determinar la disipación de lambda-cihalotrina, buprofezin, pirimetanil, tebuconazole, imidacloprid y acetamiprid en uva vinífera Sauvignon blanc y Pinot Noir, y la distribución de sus residuos en el proceso de vinificación. La vida media (TD_{50}) cuando estos plaguicidas cuando fueron aplicados en forma individual, o cuando fueron aplicados en mezcla de tanque, no presentaron diferencias y su promedio fue de 16,4; 14,0; 19,7; 26,0; 14,5 y 13,4 días para lambda-cihalotrina, buprofezin, pirimetanil, tebuconazole, imidacloprid y acetamiprid, respectivamente, sin observarse diferencia en su disipación entre ambos cultivares. Todos los plaguicidas, con excepción de lambda-cihalotrina, fueron traspasados desde la uva al vino, observándose un porcentaje de trasferencia promedio entre un 3 a 23% en el caso del vino tinto, y de un 9 a 30% en el caso del vino blanco. Los pasos que lograron una mayor reducción en la concentración de los residuos de plaguicidas en el vino tinto, fueron la fermentación alcohólica, prensado (a través del orujo) y la fermentación malolactica. En el caso del vino blanco fueron el prensado (a través de la uva y raquis), fermentación alcohólica y clarificación con bentonita. Además, en ambos vinos, el almacenado en botellas por diez meses, mostró un efecto importante en la reducción de los residuos de los plaguicidas.

Palabras clave: Carencia, factor de transferencia, residuos, vino.

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