



Preharvest Interval Periods and their relation to fruit growth stages and pesticide formulations



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ABSTRACT

The aim of this study was to evaluate the effect of pesticide formulations and fruit growth stages on the Pre-harvest Interval Period (PHI). Results showed that pesticide formulations did not affect the initial deposit and dissipation rate. However, the fruit growth stage at the application time showed a significant effect on the above-mentioned parameters. Fruit diameter increases in one millimeter pesticide dissipation rates were reduced in $-0.033 \text{ mg kg}^{-1} \text{ day}^{-1}$ ($R^2 = 0.87$; $p < 0.001$) for grapes and $-0.014 \text{ mg kg}^{-1} \text{ day}^{-1}$ ($R^2 = 0.85$; $p < 0.001$) for apples. The relation between solar radiation, air humidity and temperature, and pesticide dissipation rates were dependent on fruit type. PHI could change according to the application time, because of the initial amount of pesticide deposit in the fruits and change in the dissipation rates. Because Maximum Residue Level are becoming more restrictive, it is more important to consider the fruit growth stage effects on pesticide when performing dissipation studies to define PHI.

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1. Introduction

Although a raw product or its primary processed food can satisfy the requirements of Maximum Residue Level (MRLs), consumers demand healthy foods with non-detectable pesticide residues. However, to accomplish this important goal, the producers need specific information about the real effect of pesticide application conditions on residue dissipations, considering local productive conditions.

According to studies performed in Italy, approximately 30% of foods showed residues below MRLs, and 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; Pasarella, Elia, Guarino, Bourlot, & Nègre, 2009).

Several experimental researches conclude that factors like species, fruit growth, climatic conditions, pesticide formulation, application method, and pesticide physico-chemical properties could affect pesticide residue dissipations and therefore the residues at harvest (Balsari & Marucco, 1989; Cabras & Angioni, 2000; Cabras et al., 1997; Huo, Salazar, Hyder, & Xu, 2007; Mandal, Das, & Bhattacharyya, 2010; Marin, Oliva, Garcia, Navarro, &

Barba, 2003). However, under producer conditions or at the orchard application (commercial field application), not all of the above mentioned factors have a really high significance on dissipation processes, like fruit cultivar, formulation or application methods (Banerjee et al., 2006; Cabras et al., 2001; Pasarella et al., 2009; Alister et al., 2014; Liu, Wan, Huang, Wang, & Wang, 2012; Shirra et al., 2010). Because of this, there are still doubts about how field application factors, as pesticide formulations or application timing (fruit growth), would affect the Pre-harvest Interval Period (PHI).

For these reasons, the aims of this study were to elucidate the real effect of apple and wine grape fruits growth stage at application time on pesticide dissipations and PHI estimations, and to evaluate if the mentioned above parameters are affected by pesticide formulations.

2. Materials and methods

2.1. Pesticide field dissipation studies

The present study was performed from January to April 2014 in an orchard located at Casablanca Valley, Valparaíso region, Chile (Latitude $33^{\circ}17' \text{ S}$ and Longitude $71^{\circ}24' \text{ W}$). Apple and wine grape cultivars corresponded to Pink Lady and Sauvignon Blanc respectively. Six orchard rows of 60 m-large of each fruit species was

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selected to be treated with each selected formulated pesticide (Table 1). Two rows without pesticide application (untreated) were left as controls. Also, buffer zones between each treated and untreated row were left to avoid contamination drift. Each 60 m-long row were divided in three plots of 20 m-long to apply the selected pesticides and their respective formulations in three fruit diameter stages to apple and wine grape fruits.

The insecticides acetamiprid ((E)-N1[(6-chloro-3-pyridyl)methyl]-N'-cyano-N1-methylacetamide), formulated as Acetamiprid SL (200 g ia L⁻¹) and Hurricane 70 WP (700 g ai kg⁻¹), buprofezin ((Z)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one), formulated as Applaud 40 SC (400 g ai L⁻¹) and Buprofezin 25 WP (250 g ai kg⁻¹), and the fungicide fenhexamid (2',3'-dichloro-4'-hydroxy-1-methylcyclohexanecarboxanilide), formulated as Teldor 50 WP (500 g ai kg⁻¹) and Altivo 500 SC (500 g ai L⁻¹) were applied to apple fruits in the following diameters of fruit set: 12.1–18.4; 29.8–36.2 and 40.5–54.0 mm (Table 2) and to grape at full bloom, buckshot berries and veraison (Table 3). The application was made using an experimental turbo-nebulizer mounted to a tractor, equipped with an Albus ADR 80 nozzles, calibrated to sprayed a water volume of 695 L ha⁻¹ and 706 L ha⁻¹ to apple and vine grape plants, respectively, at 10 bar pressure. Application was performed under no wind conditions (<2 km ha⁻¹). The pesticide sprayed rates were: acetamiprid 58 g ia ha⁻¹, Buprofezin 200 g ia ha⁻¹ and fenhexamid 420 g ia ha⁻¹, independently of the formulation.

As soon as applications dried out (approximately forty min later), apple and wine grape fruit samples were collected, at 3, 10, 20, 40 and 60 days after application (DAA) for the first two application stages, and 3, 10, 20 and 40 for the last stage. This was done from each experimental plot following a random sampling for each replication (three samples for each pesticide, formulation and fruit growth stages). Samples of ±200 g were collected for grape at the initial fruit growth stage, after that ±400 g were collected. For apples ±400 g samples were collected at the initial fruit growth stages and after ±600 g. The samples were kept in plastic bags at 4 ± 1 °C until they were carried to the laboratory and maintained at -19 ± 2 °C until residue analysis. The climatic conditions at the study period were: average air temperature of 16.7 °C (8.2–27.6 °C) and relative humidity of 67.4% (30.6–96.7%). Only light rain occurred during the study (0.66 mm) and the average solar radiation was 560.8 w m⁻².

2.2. Pesticide extraction and analysis

All fruit samples were homogenized using a Grindomix® Knife Mill, and sub samples of 10 g were taken for analysis. The analysis of pesticide residues was performed using QuEChERS method. Ten grams of samples were put in 50 mL conic polypropylene tubes (Jet Biofil®) and each received 20 mL of acetonitrile (LiChrosolv® Merck). After agitation (30 min at 180 rpm) (VWR Orbital Shaker DS-500E), the polypropylene tubes with the samples were put into an Ultrasonic bath (Branson model 3510) for 10 min, and QuEChERS UCT® sachet which contains: 4g Magnesium Sulfate (MgSO₄), 0.5g of Disodium citrate (C₆H₆Na₂O₇), 0.5g of trisodium citrate (Na₃C₆H₅O₇) and 1g of Sodium Chloride (NaCl), were added to the tubes and manually shaken (10 s). All samples were centrifuged (HERMLE® Z 200A) at 4500 rpm for 5 min and after that aliquot of 10 mL was taken from each centrifuged sample and 1.5g MgSO₄ (EMSURE® ACS MerckMillipore) and 0.25g of PSA (UCT Selectra®) were added to the samples and then transferred to an ultrasonic bath for 10 min, and centrifuged at 4500 rpm for 5 min. For buprofezin and fenhexamid (Sigma-Aldrich analytical standard), an aliquot of 1.5 ml was put into a glass vial and analyzed using gas chromatography (Shimadzu Model GC-2010 Plus) with mass detector (Shimadzu GCMS-QP 2010 Ultra), equipped with a Rtx-5MS 30 m × 0.25 µm column (Restek). The gas carrier was He (Alphagaz® Helio 1Airliquide), at a flow rate of 1 mL min⁻¹ and the injector temperature was 250 °C. The samples were injected at 1 µL into the autosampler in a split-less mode, with an injection pulse of 250 kpa at 2 min. The oven temperature was: 70 °C (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). Recovery from spiked samples and retention times are shown in Table 1.

For acetamiprid (Sigma-Aldrich analytical standard), an aliquot of 5 mL of acetonitrile extract was concentrated to dryness in a rotary evaporator, re-suspended in 1.5 mL acetonitrile and transferred to a glass vial, and analyzed using high performance liquid chromatography (Hitachi LaChrom Elite Model L-2300) with diode array detector (Hitachi LaChrom Elite Model L-2450), equipped with a Kromasil® 100-5-C18 5 µm 4.6 × 250 mm column and Kromasil KR100-5C18 pre-column. The liquid phase used was water (LiChrosolv® Merck) –acetonitrile (LiChrosolv® Merck) at a flow rate of 1 mL min⁻¹ with a gradient from water-acetonitrile (95/5

Table 1
Recoveries, limit of quantification (LOQ), limits of detection (LD) and selected physic-chemical properties* of acetamiprid, buprofezin and fenhexamid.

Pesticide	Fortification levels	Recovery	LOQ	LD	Molecular weight (g mol ⁻¹)	pKa	Log _{kow}	Solubility (mg L ⁻¹ at 20 °C)	Vapor pressure (mPa)
	(mg kg ⁻¹) (n = 3) [†]	(Average ± SD)	(mg kg ⁻¹) (n = 6) [†]						
Acetamiprid (Chemical family: neonicotinoid)	0.01	93.5 ± 3.3	0.022	0.007	222.67	0.7	0.8	2,950.0	1.74 × 10 ⁻⁴
	0.05	88.2 ± 6.2							
	0.1	91.8 ± 4.0							
	0.5	93.5 ± 3.3							
	1.0	91.4 ± 2.6							
Buprofezin (Chemical family: thiadiazine)	0.01	101.0 ± 5.4	0.016	0.005	449.85	-	4.93	0.46	4.20 × 10 ⁻²
	0.05	97.7 ± 3.9							
	0.1	94.1 ± 3.3							
	0.5	96.9 ± 4.8							
	1.0	94.0 ± 1.5							
Fenhexamid (Chemical family: anilide)	0.01	89.9 ± 5.3	0.049	0.014	302.2	7.3	3.51	24.0	4.0 × 10 ⁻⁴
	0.05	93.5 ± 3.3							
	0.1	91.1 ± 2.4							
	0.5	95.1 ± 4.8							
	1.0	94.7 ± 2.2							

* Pesticide Properties Database in <http://sitem.herts.ac.uk/aeru/footprint/es/index.htm>

[†] n = number of replications.

Table 2

First order dissipation model parameters for three pesticides applied to Pink Lady apple fruits. Between parenthesis 95% confidence intervals.

Pesticides	Formulation	Fruit diameter (mm)	Parameters [†]				Residues at 40 DAA mg kg ⁻¹	R ²
			Co	k	DT ₅₀ [§]	DT ₉₀ [§]		
Acetamiprid	SL	12.1–18.4	0.645 (0.745–0.546)	0.081 (0.106–0.055)	9.2 ± 3.1	29.2 ± 9.6	0.116 ± 0.025	0.92
		29.8–36.2	0.329 (0.392–0.263)	0.064 (0.101–0.028)	14.2 ± 3.1	44.8 ± 29.8	0.033 ± 0.014	0.79
		40.5–54.0	0.230 (0.261–0.200)	0.036 (0.048–0.025)	20.6 ± 6.9	65.4 ± 21.9	0.028 ± 0.024	0.89
	WP	12.1–18.4	0.684 (0.784–0.583)	0.115 (0.129–0.068)	7.2 ± 2.6	22.7 ± 8.2	0.083 ± 0.023	0.90
		29.8–36.2	0.264 (0.302–0.225)	0.048 (0.068–0.029)	16.2 ± 7.1	51.3 ± 22.2	0.040 ± 0.013	0.86
		40.5–54.0	0.239 (0.271–0.207)	0.033 (0.044–0.023)	22.4 ± 7.6	70.9 ± 24.2	0.036 ± 0.016	0.87
Buprofezin	SC	12.1–18.4	0.641 (0.728–0.555)	0.102 (0.131–0.078)	7.0 ± 1.8	22.1 ± 5.7	0.030 ± 0.014	0.91
		29.8–36.2	0.228 (0.250–0.206)	0.051 (0.065–0.037)	14.4 ± 4.1	45.6 ± 12.9	0.015 ± 0.009	0.98
		40.5–54.0	0.301 (0.328–0.275)	0.102 (0.127–0.076)	7.2 ± 1.9	22.6 ± 5.8	0.010 ± 0.006	0.97
	WP	12.1–18.4	0.706 (0.774–0.639)	0.132 (0.160–0.103)	5.4 ± 1.9	17.2 ± 3.8	0.039 ± 0.016	0.96
		29.8–36.2	0.218 (0.244–0.192)	0.046 (0.061–0.031)	16.3 ± 5.6	51.5 ± 17.7	0.027 ± 0.004	0.91
		40.5–54.0	0.276 (0.318–0.233)	0.145 (0.209–0.081)	5.6 ± 2.7	17.6 ± 8.6	0.008 ± 0.003	0.91
Fenhexamid	SC	12.1–18.4	4.100 (4.364–3.837)	0.187 (0.220–0.154)	3.8 ± 0.7	12.0 ± 2.1	0.142 ± 0.04	0.98
		29.8–36.2	2.265 (2.547–1.982)	0.091 (0.125–0.056)	8.5 ± 3.5	27.0 ± 11.1	0.092 ± 0.018	0.93
		40.5–54.0	2.666 (2.859–2.473)	0.084 (0.101–0.068)	8.4 ± 1.8	26.7 ± 5.3	0.036 ± 0.008	0.98
	WP	12.1–18.4	3.872 (4.127–3.617)	0.161 (0.187–0.134)	4.4 ± 0.7	13.9 ± 2.3	0.232 ± 0.048	0.98
		29.8–36.2	2.161 (2.411–1.911)	0.054 (0.070–0.037)	13.8 ± 4.5	43.8 ± 14.2	0.398 ± 0.115	0.92
		40.5–54.0	2.442 (2.724–2.160)	0.075 (0.098–0.052)	9.8 ± 3.2	31.3 ± 10.1	0.127 ± 0.061	0.94

[†] Co = Initial fruit pesticide concentrations (mg kg⁻¹); k = One order dissipation constant (days⁻¹); DT₅₀ and DT₉₀ = 50% and 90% dissipation time (days).[§] Values are means of three replications ± standard deviation.**Table 3**

First order dissipation model parameters for three pesticides applied to Sauvignon blanc vine grape berries. Between parenthesis 95% confidence intervals.

Pesticides	Formulation	Fruit diameter (mm)	Parameters [†]				Residues at 40 DAA mg kg ⁻¹	R ²
			Co	K	DT ₅₀ [§]	DT ₉₀ [§]		
Acetamiprid	SL	1.6–2.1	2.298 (2.657–1.945)	0.410 (0.592–0.227)	2.0 ± 0.9	6.3 ± 3.1	0.182 ± 0.030	0.91
		6.2–11.0	0.899 (1.025–0.773)	0.205 (0.287–0.122)	3.8 ± 1.7	12.1 ± 5.3	0.069 ± 0.004	0.92
		11.8–18.3	0.276 (0.327–0.266)	0.091 (0.130–0.051)	8.8 ± 4.2	28.0 ± 13.4	0.015 ± 0.020	0.88
	WP	1.6–2.1	1.861 (2.094–1.628)	0.457 (0.627–0.287)	1.8 ± 0.7	5.3 ± 2.1	0.118 ± 0.027	0.94
		6.2–11.0	0.909 (1.017–0.803)	0.221 (0.298–0.145)	3.4 ± 1.3	10.8 ± 3.9	0.058 ± 0.010	0.94
		11.8–18.3	0.300 (0.356–0.245)	0.056 (0.081–0.031)	14.5 ± 7.3	46.1 ± 23.3	0.025 ± 0.018	0.85
Buprofezin	SC	1.6–2.1	2.676 (2.876–2.476)	0.394 (0.479–0.309)	1.8 ± 0.4	5.7 ± 1.3	0.018 ± 0.009	0.98
		6.2–11.0	1.066 (1.192–0.940)	0.246 (0.335–0.158)	3.1 ± 1.2	9.8 ± 3.8	0.042 ± 0.020	0.95
		11.8–18.3	0.299 (0.359–0.240)	0.122 (0.181–0.063)	6.8 ± 3.7	21.7 ± 11.8	0.041 ± 0.014	0.85
	WP	1.6–2.1	2.434 (2.665–2.204)	0.297 (0.381–0.214)	2.5 ± 0.7	7.8 ± 2.3	0.023 ± 0.013	0.97
		6.2–11.0	1.054 (1.194–0.913)	0.283 (0.406–0.160)	2.8 ± 1.4	8.9 ± 4.3	0.072 ± 0.030	0.93
		11.8–18.3	0.334 (0.400–0.269)	0.058 (0.086–0.030)	14.3 ± 7.6	45.4 ± 24.3	0.077 ± 0.023	0.80
Fenhexamid	SC	1.6–2.1	3.328 (3.641–3.016)	0.324 (0.412–0.235)	2.3 ± 0.6	7.2 ± 2.0	0.177 ± 0.042	0.96
		6.2–11.0	1.938 (2.110–1.766)	0.049 (0.060–0.038)	14.7 ± 3.3	46.5 ± 10.5	0.331 ± 0.105	0.96
		11.8–18.3	0.968 (1.128–0.807)	0.045 (0.062–0.028)	17.4 ± 7.2	55.0 ± 22.6	0.214 ± 0.092	0.87
	WP	1.6–2.1	3.142 (3.537–2.748)	0.296 (0.406–0.186)	2.6 ± 1.0	8.2 ± 3.3	0.263 ± 0.107	0.93
		6.2–11.0	2.095 (2.201–1.829)	0.050 (0.061–0.038)	14.4 ± 3.4	45.8 ± 10.8	0.317 ± 0.101	0.96
		11.8–18.3	1.086 (1.231–0.940)	0.052 (0.069–0.034)	14.6 ± 5.1	46.2 ± 16.2	0.239 ± 0.100	0.90

[†] Co = Initial fruit pesticide concentrations (mg kg⁻¹); k = One order dissipation constant (days⁻¹); DT₅₀ and DT₉₀ = 50% and 90% dissipation time (days).[§] Values are means of three replications ± standard deviation.

v/v) for 1 min, to 85/15 (v/v) during 3 min, kept at 85/15 (v/v) for 4 min, to 20/80 (v/v) during 9 min, to 95/5 (v/v) during 10 min, and kept for 2 min at 95/5 (v/v). The column temperature was 35 °C and the injection volume was 20 µL. The detector (Hitachi model Elite LaChrom L-2450) conditions and recovery from spiked samples are shown in Table 1.

In both quantification techniques (Gas and Liquid chromatography) was used a Procedure Standard Calibration as an alternative type of calibration, to compensate for matrix effects and low extraction recoveries associated with certain pesticide/matrix combinations, according to the European Commission (2015). Procedural standards were prepared by spiking a blank portion of both matrix (apple fruits and grapes berries) prior to extraction, with five-pesticide concentration levels and three replications, and then analyzed in exactly the same way as the study samples.

2.3. Data analysis

Results of pesticides residues, in grapes berries and apple fruits, were analyzed fitting to a first order kinetic model [1], using non-linear regression analysis, to determine pesticide dissipation parameters, defined for the following equations:

$$C = C_0 * \exp(-k * t) \quad (1)$$

$$DT_{50} = \ln 2 / k \quad (2)$$

$$DT_{90} = \ln 9 / k \quad (3)$$

where C (mg kg⁻¹) is the apples fruit or grape berries pesticide concentrations at time t (days), C₀ (mg kg⁻¹) is the initial pesticide concentration, and k (1 day⁻¹) is a one order dissipation rate, that determines the slope of the curve. The model prediction capacity

was calculated using r-square (R^2). The 50% dissipation time (DT_{50}) and 90% dissipation time (DT_{90}) were estimated using Eqs. (2) and (3), respectively.

The Pearson correlation procedure was used to determine the relation between selected climatic conditions (Free water, solar radiation, air relative humidity, air temperature and rain) and application conditions (initial deposit and equatorial fruit diameter) to dissipation rates (k), to each selected pesticide, combined the result from both formulations, because the analysis of means do not show any significant effect on dissipation rate (k) or initial deposit.

3. Results and discussion

3.1. Relation between fruit diameter, formulation and initial deposit

Results showed a clear effect of fruit diameter on pesticide residue concentrations; however the effect was more significant in the case of grape berries than in apple fruits (Fig. 1). Very similar results were obtained in a preliminary work, during the previous season, in the same orchard with acetamiprid WP and buprofezin SC (Data not published). The initial pesticide deposit in apple fruit and grape berries varied according to fruit diameters at application time, independently of pesticide and formulations. Thus, grape berries showed around 56% reduction in pesticide residues, when their equatorial diameter varied from 2.1 to 9.3 mm, and 82% when increased to 18.3 mm. In apple fruits a significant reduction in deposit was seen when equatorial diameter increased from

approximately 15 mm to 30 mm (Stage 1 to 2), but this effect was not observed when pesticides were applied to fruits larger than 30 mm diameter (Fig. 1 and Tables 2 and 3). These results were concordant with other researchers whom have shown that pesticide deposit is affected by the fruit growth stage (Balsari & Marucco, 1989; Huo et al., 2007).

Other works suggest that other factors, as water volume, canopy architecture and surface structure also could affect the initial deposit (Ade, Pezziet, Cooper, & Taylor, 2000; Angioni, Porcu, & Dedola, 2012; Bruhn & Fry, 1982; Hall, Downer, Cooper, Eber, & Ferree, 2007; Wise, Jenkins, Schilder, Vandervoort, & Isaacs, 2010). However, in our study the water volume and canopy architecture (fruit exposure) were maintained constant during the application stages.

Results from the literature are not conclusive respect to the real effect of different pesticide formulations in residue deposits. Thus, Gupta and Gajbhiye (2011), sprayed different cypermethrin formulations to tomato fruits, did not find significant difference in deposits, even using rates that varied from 40 to 80 g ai ha⁻¹. Angioni et al. (2011) applied three formulations of chlorpyrifos (EC, WG and ME) to orange, peach, tomato fruits and grape berries. Their results showed no initial deposit difference in orange and tomato fruits, but in peach fruits residues deposit was larger with EC respect to WG and ME formulations. Similarly, Zhou et al. (2014) did not find differences when rotenone was applied to cabbage, formulated as SC or WDG.

3.2. Pesticide dissipation and PHI

Several studies have showed that pesticide residue dissipations in horticultural products (i.e: peach, grape berries, tomato fruit, lettuces, etc), show a higher dissipation rates during the first week after application, followed by a slow rate during the next weeks (Alister et al., 2014; Cabras et al., 2001; Galiotta et al., 2011; Liang et al., 2012; Pasarella et al., 2009; Valverde-García, González-Pradas, & Aguilera-del Real, 1993). For these reasons the use of $n > 1$ order exponential model did not show a significant improvement for the goodness of fit. Thus, the results for the three application growth stages (Tables 2 and 3) are well described by a first order exponential model

The regression analysis showed a lineal relationship between fruit diameter increases and dissipation rate reductions (k). When the fruit diameter increased by one millimeter dissipation rate was reduced to $-0.033 \text{ mg kg}^{-1} \text{ day}^{-1}$ ($R^2 = 0.87$; $p < 0.001$) and $-0.014 \text{ mg kg}^{-1} \text{ day}^{-1}$ ($R^2 = 0.85$; $p < 0.001$), for grape berries and apple fruits, respectively. These results showed that variation in grape berry diameters is more significant for residue dissipations than in apple fruits. Some authors, as Thorbek and Hyder (2006) considered that 50% of pesticide dissipations is related to their physico-chemical properties, however other authors consider that fruit growth stage could affect in an important way pesticide dissipation curves (Banerjee et al., 2006; Bouri et al., 2012). This later view is concordant with the results of the present study.

In Tables 2 and 3, the residues at fourteen days after applications (DAA) were compared, which corresponded to the last sampling date for the third application growth stages. The residues of all pesticides showed important concentration levels and non-effect of formulations was determined (Tables 2 and 3). Regardless of this, the literature shows contrasting results respect to the real effect of formulation in pesticide residue dissipations. Thus, Angioni et al. (2011) found differences in chlorpyrifos DT_{50} values when they compared ME to EC and WG formulations, but residue concentrations at 45 DAA were independent of pesticide formulation in orange fruits. In peach fruits chlorpyrifos residues at 39 DAA were higher for ME than EC and WG formulations. However, in table grape berries the DT_{50} for ME and EC formulations were sim-

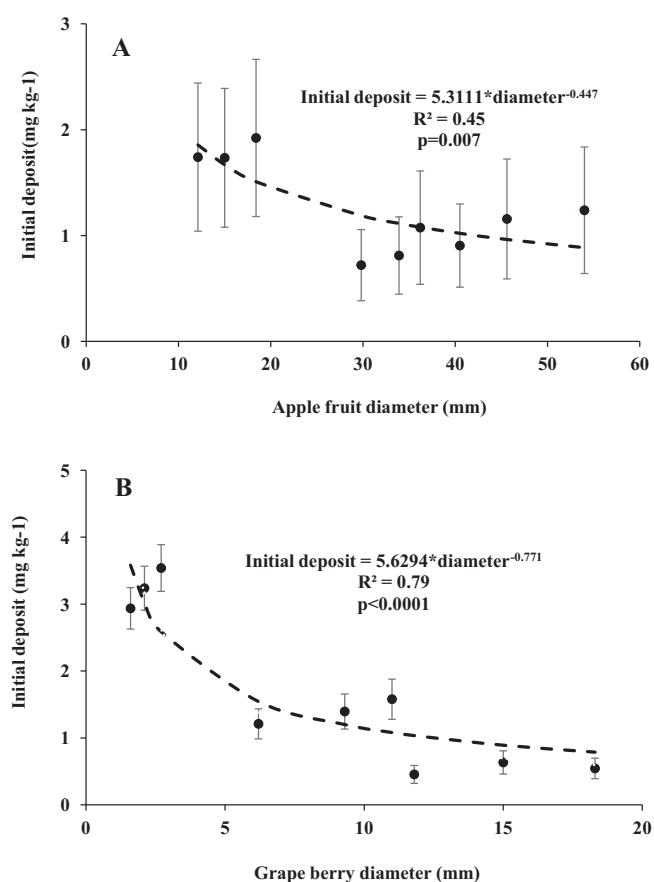


Fig. 1. Relation between apple (A) and grape berry (B) diameter and pesticide initial deposit. Dark circles correspond to an average of six replication of quantified residues and error bars to standard error of the means. Segmented line corresponded to exponential model.

Residues of fenhexamid were more persistent in comparison to acetamiprid and buprofezin, in the second and third fruit growth stage applications (Tables 2 and 3). These results do not agree with the few existing published data, which came from other species and showed reported half-lives between 4.7 and 5.0 days for pepper and strawberry fruits, respectively (Angioni et al., 2004; Hem et al., 2011) and approximately 2.0 days for wine grape berries (Cabras et al., 2001). Malhat, El Sharkawi, Loutfy, and Ahmed (2014) determined residues below 0.3 mg kg^{-1} in grapes berries, at 21 DAA, and DT_{50} of 4.21 days using a similar rate of fenhexamid than the applied in the present study. The DT_{50} was different from the one obtained in this study, which was around 14 days (Table 3). The smallest dissipation rate observed in our study could be explained because of the climatic conditions, especially temperature and relative humidity, because the average air temperature was around 22°C in comparison to the reported studies in which temperatures varied from 28 to 30°C , with a relative humidity larger than 70% (Hem et al., 2011; Malhat et al., 2014). Moreover, Malhat et al. (2014) explained the fast fenhexamid residue dissipation because the interaction between humidity and solar radiation. However, in the present study this relation between climatic parameters and fenhexamid dissipation was dependent on fruit type. Thus, air temperature was inversely correlated to fenhexamid dissipation rate in apple fruit but in grape berries was directly correlated (Table 4).

This interaction between pesticide dissipation and fruit type was also observed for acetamiprid and buprofezin dissipation. For example, solar radiation, relative humidity and air temperature show high correlation coefficients for acetamiprid and buprofezin in grape berries, but not in apple (Table 4). However, in almost all studied pesticides and fruits (apple and grape), fruit diameter at application time and initial deposit were highly correlated to dissipation rate (Table 4).

All pesticides showed detectable residues until the end of the study period for each growth application stage. The PHI was estimated (Table 5) considering the MRLs establish in the European Union or USA for each pesticide which were replaced for the term C (Residue concentration at time t) into the dissipation model (Eq. (1)), and using the dissipation rate for each fruit growth stage (Tables 2 and 3). Fenhexamid in vine grape berries or acetamiprid and buprofezin in apples presented a PHI less than one day to accomplish their respective MRLs, independent of the fruit growth stage. However, when the MRLs are more restricted, the timing of application acquired a significant importance. Thus, when fenhexamid was applied to small diameter apple fruits or acetamiprid applied to small wine grape berries, the PHI should be reduced in comparison to the later applications (Stage of application 2 and 3), because the dissipation rate was larger due to the rapid increase of fruit diameter (Table 2, 3 and 5).

4. Conclusions

Fruit growth stages at the time of pesticide applications (Fruit equatorial diameter), would affect the initial deposit and pesticide dissipation rates. However, pesticide formulations (SC, SL and WP) would not have a significant effect on initial deposit and/or dissipation rates for the studied pesticides. The climatic conditions, as solar radiation, relative humidity and air temperature could be important factors to consider for pesticides dissipation rates, but their effect could depends of fruit type.

Pre-harvest interval could change because of the application stages, due pesticide deposits in the fruits and changes in the dissipation rates, but the importance of these factors will be related to the MRLs to be accomplished. As soon as MRLs became more

restrictive, the fruit growth stage at application time will be more important in order to define a PHI.

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