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Bagging Technology Reduces Pesticide Residues in Table Grapes

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Abstract

Most table grape growing regions in China have a continental climate. This climate is conducive for grape diseases. For this reason, our research group developed a bagging technology and a design product twenty years ago to protect the berries from damages by pests. This innovation was granted a patent in 2002 in China. Since then, this patent product has been dramatically extended to grape growers in China. Grape bagging is currently one of the most widely used viticultural practices in the table grape industry in China. However, effects of grape bagging on reduction of pesticide residues were not comprehensively evaluated. In this study, residues of seven pesticides, omethoate, cyhalothrin, mancozeb, methylthiophanate, chlorothalonil, metalaxyl mancozeb and triadimefon, were compared between the bagged and nonbagged berries of the two popular grape cultivars, 'Red Globe' and 'Kyoho', planted in China. The results showed that an annual program of 25 - 30 and 17-20 of pesticide applications were needed in Yangling, a typical region for growing table grapes in China to control diseases in 'Red Globe' and 'Kyoho', respectively. The recovery of the seven pesticides was over 96% and the precision of the determining method (RSD) was around 7% in most cases, indicating that the analytical methods were adequate. Pesticide residues in both of the nonbagged and bagged berries were lower than the grape maximum pesticide residue limits required by China government. The pesticide residues in the bagged berries were reduced to ~10 % of the residues in the nonbagged berries. This indicates that the bagging technology extensively reduced the pesticide residues in the berries.

Both production and planted area of table grapes in China are the largest among the countries in the world (He, 1999; Wan et al., 2008). The planted area and production were 715 kilo hectors and 11.6 million tons in 2013, respectively (FAO, data released in 2015). The table grape is one of the most favorite fruits appealing to both growers and consumers in China because of its short juvenility, relatively low cost and high return, high quality and rich nutrition in berries (He, 1999; Wan et al., 2008). However, most regions suitable for growing table grapes in China have a continental climate with the major rainy season from April to Oct., which is concurrent with the growing season (Wan et al., 2007; 2008). This climatic feature usually results in a high risk of disease epidemics in most grape production regions in China (Wan et al., 2007). As a result, a fungicide spray per week is suggested to the growers to control diseases in these regions and an annual estimation of 10 kilo tons of fungicides is needed to control the diseases for table grapes in China (Xiong et al., 2002a and 2002b; Li et al., 2012). Heavy reliance of pesticides for table grape production resulting in high pesticide residue in both of the vineyard and the grapes is a severe global environmental problem (Soles and Goldberg, 2000; De Melo Abreu et al. 2006; Dehouck et al., 2015). The pesticide residue in table grapes may be an even worse risk to consumers because some consumers eat the grapes directly without any processing, e.g. washing and scalding, to minimize the

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pesticide residues before eating (Turgut et al., 2011; Grimalt and Dehouck, 2016).

Bagging viticulture has been successfully extended to the growers in China in the past decade (Wan, 2002; Zhou and Guo, 2005; Zheng et al. 2014). It is estimated that this technology is applied to 80% of the table grapes (~ 230,000 hectors in 2013) in China (Zheng et al. 2014). This is the first report to compare pesticide residues in bagged and nonbagged berries of the two most popular grape cultivars in China. This report will be very informative to the grape growers in other countries when they plan to apply this technology in their vineyards in the future.

Materials and Methods

Plant materials. Both 'Red Globe' and 'Kyoho' plants were obtained from the Experiment Station of Northwest A&F University, Yangling, Shaanxi, China. The grapevines were 10-12 years old, self-rooted and planted in the vineyard at a spacing of 1.2 × 2.5 m.

Bagging treatment and harvest. High quality paper bags (made by Tianjing Agriculture Preservation Technology Research Institute, Tianjing, China) were used for bagging. The twist wire was embedded near the mouth of the bags for efficient bagging (Wan 2002). The bags were made of the kraft white and soft paper without any coating on the paper. Holes were made in the bottoms of the bags for aeration and drainage. Prior to bagging, the grapevines were completely sprayed with chlorothalonil on a sunny day four weeks post full bloom using the normal pesticide spray method. After the spray, the berry bunches were promptly bagged after the clusters were dried. The nonbagged berry bunches used as the control were also sprayed with chlorothalonil at the same time. Bagging started on 20 June (at stage 31, with berries pea size), and ended 10 days before the harvest facilitating berry coloration (Wan 2002; Fig 1). After bag removal and before harvest, vines were not sprayed with pesticides. 'Kyoho' was harvested in the end of Aug., and 'Red Globe' in the end of Sept. from 2013 through 2015.

Pesticide sprays. Yangling has a typical continental climate. Vines were sprayed

Fig. 1. Bagged clusters with the bags pulled up 10 days before harvest for berry coloration.

every seven and 10 days to control pests in 'Red Globe', and 'Kyoho', respectively

Assessment of pesticide residues. Berries were harvested when fully ripened by berry color and taste. About 100 g of berries were randomly chosen from each of 10 bunches (one bunch from each of 10 vines) for one experiment. There were three biological replicates, where the experimental unit was a 10-vine section of row. The standard chemicals and other chemicals were bought from Sigma-Aldrich Co. (St. Louis, Missouri, USA).

Determination of mancozeb was based on the protocol by head space gas chromatography (Lin et al., 2013), in which mancozeb was transferred into carbon disulfide. The chemical transformation of mancozeb into carbon disulfide was carried out in the Head Space Injector (Chengdu Colintech Analysis Co., Chengdu, China) (Lin et al., 2013).

Determination of omethoate and metalaxyl mancozeb was carried out using Agilent 7890 Gas Chromatography plus a flame photometric detector with a column of DB-B 30 m × 0.22 mm × 0.35 μm (GC-FED, Santa Clara, CA, USA) (Li et al., 2012). Determination of cyhalothrin, chlorothalonil and triadimefon was using Agilent 7890 Gas chromatography plus an electron capture detector with a column of DB-B 30 m×0.22 mm×0.35 μm (GC-ECD, Santa Clara, CA, USA). Determination of methylthiophanate was done using Agilent 1100 High Performance Liquid Chromatography (HPLC, Santa Clara, CA, USA) (Li et al., 2012).

The recovery tests were carried out on six replicates (Pizzuttia et al., 2009).

Randomized block experiment design was used for this study. Three 10- vine plots from each of three blocks within the vineyard were used for each treatment, repeated for three years, were used for determination of a pesticide residue. Data were averaged from three blocks of a year and one-way ANOVAs were performed with a year as 'a replicate', using SPSS v13.0, for each combination of cultivar and pesticide to compare bagging treatments

Results

Frequency of the pesticides used in Yangling. The annual precipitation is 700-900 mm in Yangling, and about 65% of the yearly

Table 1. The pesticide frequency used in the growing season for control of pests in the two grape cultivars of 'Red Globe' and 'Kyoho' in this study.

Pesticides	Control of the major pests	Frequency of pesticides (g/hectare)	
		Red Globe	Kyoho
Omethoate	Mites, aphids, planthoppers, leafhoppers	Twice or three times, 1200-1800g	Once or twice 600-1200g
Cyhalothrin	Thrips, aphids,	Once, 600g	Once, 600g
Mancozeb	Downy mildew, elsinoe anthracnose, white rot, black rot. brown rot	Five or six times, 3000-3600g	Three times, 1800g
Methylthiophanate	Anthracnose rot, white rot, grey mildew, brown rot	Five to seven times, 3000-4200g	Four or five times 2400-3000g
Chlorothalonil	Powdery mildew, elsinoe anthracnose, anthracnose rot, berry rot	Five to seven times, 3000-4200g	Four or five times, 2400-3000g
Metalaxyl mancozeb	Downy mildew, white rot, anthracnose rot	Four times, 2400g	Three times, 1800g
Triadimefon	Powdery mildew	Three times, 1800g	Once, 600g

rainfall concentrates from 20 May to the end of Sept. (Wan et al., 2008). 'Red Globe' is a cultivar derived from *Vitis vinifera*; and 'Kyoho' from a hybrid of *Vitis labrusca* × *Vitis vinifera*. 'Red Globe' usually ripens in late Sept. or early Oct., and 'Kyoho' in late Aug. in Yangling. The species of *Vitis vinifera* is more susceptible to fungal pests than *Vitis labrusca* (He, 1999; Wan et al., 2008). Thus, 'Red Globe' is much more susceptible to fungi than 'Kyoho'. In addition, the berry growing period of 'Red Globe' is much longer than that of 'Kyoho'. For these two reasons, pesticides are applied more frequently to 'Red Globe' than 'Kyoho' (Table 1). Usually, 17 to 20 sprays are needed for disease control in 'Kyoho', and 25 to 30 sprays are needed for 'Red Globe' per season in Yangling.

For most of the fungicides, over 65% of the chemical functions were degraded within two days after spray (Li et al., 2012; Dehouck et al., 2015). The high moisture following a rain event potentially increases disease infection in the berries and vines. Thus, a prompt fungicide application following rain is suggested in this growing region and more pesticides may be used than in Table 1 for Yangling in some years with high rainfall.

Mean recovery and relative standard deviation (RSD.) To evaluate the recovery of the method, analyses were carried out in six replicates of "blank" grape samples at three different levels (20, 50 and 500μg/kg) and its RSD was calculated.

As shown in Table 2, the recovery was over 96% and RSD was around 7% in most cases, suggesting the analytical method in this study was reliable and its precision was

Table 2. Mean recovery and relative standard deviation (RSD) for determination of the seven pesticides

Pesticides	Standard concentrations	Recovery (%)	RSD (%)
Omethoate	20	101.8	6.6
	50	97.5	11.3
	500	96.3	5.7
Cyhalothrin	20	112.6	7.2
	50	96.6	12.6
	500	98.2	6.2
Mancozeb	20	103.2	7.3
	50	98.7	12.8
	500	99.4	6.9
Methylthiophanate	20	102.6	7.1
	50	97.3	11.6
	500	98.6	6.5
Chlorothalonil	20	105.6	7.3
	50	99.2	12.6
	500	98.4	6.5
Metalaxyl mancozeb	20	101.7	7.0
	50	97.6	10.6
	500	98.4	6.6
Triadimefon	20	106.2	6.5
	50	99.3	12.6
	500	98.7	7.2

Table 3. Pesticide residues (μg/kg) in the bagged and nonbagged berries of two grape cultivars[z].

Cultivars	Pesticides	Pesticide residues										
		2013	2014	2015	Avg.	S.D.[y]	2013	2014	2015	Avg.	S.D.	p values [w]
Red Globe [x]	Omethoate	ND	ND	ND	-	-	ND	ND	ND	-	-	-
Cyhalothrin		ND	ND	ND	-	-	16.4	17.7	18.3	17.5	1.0	- or very low
	Mancozeb	12.7	16.2	13.8	14.2	1.8	154.3	163.4	172.3	163.3	9.0	9.49×10^{-6}
	Methylthiophanate	12.8	16.8	13.1	14.2	2.2	134.3	148.6	126.7	136.5	11.1	4.84×10^{-5}
	Chlorothalonil	17.4	14.3	18.4	16.7	2.1	190.6	187.3	178.3	185.4	6.4	1.67×10^{-6}
	Metalaxyl mancozeb	47.6	64.5	50.8	54.3	9.0	187.6	166.8	174.5	176.3	10.5	1.07×10^{-4}
	Triadimefon	10.5	12.8	13.7	12.3	1.7	144.4	156.3	147.6	149.4	6.2	3.10×10^{-6}
Kyoho [x]	Omethoate	ND	ND	ND	-	-	ND	ND	ND	-	-	-
	Cyhalothrin	ND	ND	ND	-	-	10.2	11.6	12.7	11.5	1.3	- or very low
	Mancozeb	8.6	6.3	9.5	8.1	1.7	90.7	78.6	80.3	83.2	6.6	4.29×10^{-5}
	Methylthiophanate	12.6	9.7	11.2	11.2	1.5	106.7	120.5	97.6	108.3	11.5	1.33×10^{-4}
	Chlorothalonil	12.5	10.3	14.6	12.5	2.2	130.5	112.8	117.9	120.4	9.1	3.71×10^{-5}
	Metalaxyl mancozeb	8.6	6.2	7.8	7.5	1.2	50.3	55.7	60.2	55.4	5.0	8.41×10^{-5}
	Triadimefon	10.2	11.8	13.6	11.9	1.7	132.5	126.3	147.2	135.3	10.7	3.93×10^{-5}

Note: [z] One-way ANOVA was performed for each combination of pesticide and cultivar
[y] S.D. was the standard deviation from the average values of three years' data.
[x] P-value for difference of pesticide residue in the bagged berries between two cultivars was 0.01075, and P-value was 0.004444 for the nonbagged berries. For this analysis, two cultivars were used as 'factors'.
[w] P- values for residue differences between the bagged and nonbagged berries.

relatively high (Dehouck et al., 2015; Grimalt et al., 2016).

Pesticide residues in the real berry samples. For the bagged or nonbagged berries, the pesticide residues in 'Red Globe' were significantly higher than those in 'Kyoho' ($p < 0.02$) (Table 3). However, the pesticide residues in both treatments and cultivars in this study (Table 3) were lower than the grape maximum pesticide residue limits made by the China government and the Codex Alimentarius Commission (Shu et al., 2005; Yang et al., 2007). The pesticide residues in the nonbagged berries were ten fold higher than for bagged berries in most cases (Table 3), indicating the bagging technology was able to extensively reduce the pesticide residues in the berries ($p < 0.001$). However, the reduction differed among the pesticides. The reduction of metalaxyl mancozeb in 'Red Galobe' was relatively small by bagging compared to other pesticides. But the reason for this phenomenon is unclear.

Discussion and Conclusion

Establishment of reliable analytical methods is prerequisite to precisely determine pesticide residue in grapes. In the past two decades, Chinese viticulturists surveyed reliable methods to determine pesticide residue in grapes (Li et al., 2012). The standard methods for analysis of pesticide residues in agriculture have been established and published in government documents in China. This study was performed according to these standard methods. Our results were compatible with previous reports (Pizzuttia et al., 2009; Li et al., 2012).

Fifteen years ago, the bagging technology started to extend to Chinese grape growers (Wan, 2002; Zhou and Guo, 2005; Zheng et al. 2014). Currently, over 80% of the table grapes are bagged and the technology is important to improve berry quality (Zheng et al. 2014) and berry appearance (Wan, 2002; Zheng et al., 2014). Little dust is deposited in the bags (Fig 1), thus the appearance of the bagged berries was brighter than nonbagged berries and the brighter appearance appeals to consumers (Zheng et al., 2014).

Most of the pesticide residues in 'Red Galobe' were much higher than those in 'Kyoho', particularly in nonbagged berries (Table 3). As aforementioned, 'Red Globe' is more susceptible to disease than 'Kyoho' (Wan et al., 2008). The berry growth period of 'Red Globe' was longer than for 'Kyoho'and at harvest 'Red Globe' usually is severely infected with downy mildew in Yangling (Wan et al., 2008). For these reasons, pesticides were more frequently used in 'Red Globe' than 'Kyoho', possibly resulting in a high pesticide residue in 'Red Globe' (Table 3). However, other factors, such as berry size or cluster compactness, may also cause differences between cultivars.

The results of this study reflect the pesticide residues in the whole berries. However, the pesticide residues in the berry skins should be higher than those of the entire fruits. Washing can remove most of the pesticide residues on the berry skins (Zheng et al., 2014). Thus, consumers are still advised to wash grapes before eating, though trace of pesticide residues was deposited on the bagged berries (Wan, 2002; Zheng et al., 2014).

In conclusion, the berry bagging technology is currently a popular viticulture practice used in the Chinese table grape industry and greatly decreased the pesticide residues in the grape berries.

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