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Spatial Distribution of Ochratoxin A in Vineyard and Sampling Design To Assess Must Contamination

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ABSTRACT

The aim of this work was to study the spatial variability of ochratoxin A (OTA) in vineyards and to define a reliable sampling protocol for bunches in order to assess OTA content before harvesting. In 2002, two vineyards with 'Negroamaro' and 'Sangiovese' grape varieties were chosen in Southern Italy. The same sampling design was applied to both vineyards. Ten plants were collected from the X-shaped path of the whole vineyard (plants 1 through 10) and 10 plants from the two central cross-perpendicular lines (plants 11 through 20). Bunches harvested from plants 1 through 10 were numbered progressively, weighed, and crushed separately, and bunches obtained from plants 11 through 20 were crushed plant by plant. Juices obtained were analyzed for OTA content by high-performance liquid chromatography. Then, a simulated approach for sampling was applied, following randomized and systematic designs. High and random variability was observed in OTA content both among bunches and among plants in the two vineyards, independent of contamination level. Simulated sampling design markedly influenced the assessment of must contamination. The best results were obtained when sampling involved one bunch per plant, in a predefined position, from at least 10 plants. Estimated means, obtained with different sampling designs, did not differ significantly from calculated mean OTA content. If the true contamination in a vineyard is 2 μg kg^{-1}, the limit fixed in Europe for OTA content in must and wine, the range of OTA content assessed sampling one bunch in the central position of 10 plants should lie between 2.9 and 1.4. The accuracy could be considered acceptable.

Quantification of mycotoxins in foods and feeds is a relevant issue because of the risk of harmful effects on human and animal health and the importance of making correct decisions when a lot has to be classified as legally acceptable or unacceptable.

A mycotoxin test procedure is a multistage process and generally consists of three steps: sampling, sample preparation, and analysis. A lot of work has been done on mycotoxin analytical methodology *(1)*, although limited efforts have been devoted to sampling procedures. However, the sampling step is crucial; in fact, it is usually the largest source of uncertainty associated with a mycotoxin test procedure because of the spot distribution of contaminated particles within lots *(15, 23)*.

The mycotoxin concentration of a lot is usually estimated by measuring its content in a small sample taken from the lot, and the value is assumed to be representative of the whole lot. If the sample concentration does not accurately reflect the lot concentration, the lot may be misclassified and there may be undesirable economic and/or health consequences *(23)*. Specific studies have been managed to design sampling plans for the determination of aflatoxins in maize and peanuts *(18, 20)*, of deoxynivalenol in wheat *(22)*, or for a general approach regarding mycotoxins in foods and feeds *(7)*. All these studies examine mycotoxin quantification procedures postharvest. Only one attempt has been made to predict aflatoxin level from field data, but it was based on fungi quantification in soil *(14)*.

Mycotoxin production is a complex phenomenon depending on many interacting factors. The adoption of a holistic system approach to the surveillance of mycotoxins facilitates an analysis of the many interacting components of the system and, subsequently, the identification of those constraints within the system that are leading to the onset of spoilage and, ultimately, to the production of mycotoxins. Consequently, sampling plans that ensure that the analytical data generated identify those key points (critical points) that are contributing to the spoilage of the commodity and to the production of mycotoxins are required *(7)*. Most mycotoxin problems originate in the field, and therefore mycotoxin control must start during crop growing.

Grape has been included among potential sources of ochratoxin A (OTA) exposure only since 1996, when Zimmerli and Dick *(25)* detected the mycotoxin in wine for the first time. Further research found that the contamination originated in the vineyard *(4)*. Even if OTA in grapes is a recent problem, Commission Regulation no. 123/2005 established a maximum level of 2.0 μg kg^{-1} for OTA in wine and grape juice *(8)*. Therefore, it is important to establish sampling protocols for vineyards.

The aim of this work was to study the spatial variability of OTA in grape vineyards and to define a reliable sampling protocol for grape bunches to assess OTA content before harvesting. A correct quantification of OTA in vine-

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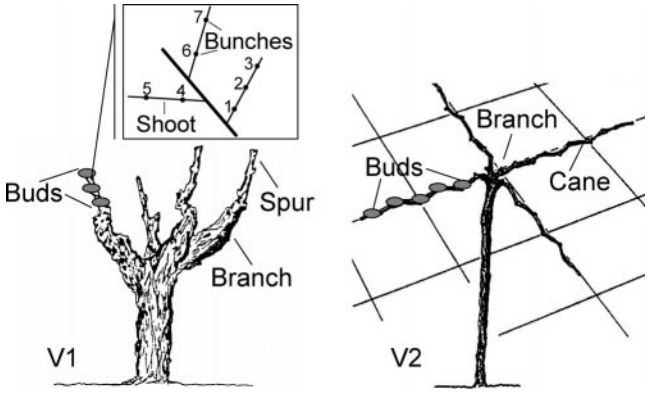

FIGURE 1. *Basic structure of 'Alberello' in vineyard 1 (V1) and 'Tendone' in vineyard (V2) training systems. 'Alberello' has four main branches, each one with one spur, pruned with three buds. In this example, one spur with three shoots developed from the three buds, with three, two, and two bunches, respectively, numbered 1 through 3 on the basal shoot, 4 and 5 on the following, and 6 and 7 on the apical shoot highlighted as example. 'Tendone' has four branches, each with one cane, pruned with five buds (modified from (13)).*

yards would enable a good estimate of its level in must obtained from the crushing of bunches in wineries. The advantage would be that if the content of OTA was known before harvesting, the wine-making process could then be modified accordingly. Operations could be adapted appropriately in cases where a high content of the contaminant is detected.

MATERIALS AND METHODS

OTA content in bunches: sampling of bunches. In 2002, two vineyards in San Pancrazio Salentino (Brindisi, Southern Italy) were chosen. Grape varieties 'Negroamaro' and 'Sangiovese' were cultivated in vineyard 1 (V1) and in vineyard 2 (V2), respectively. V1 consisted of 84 rows, with 130 plants along each row, while V2 had 22 rows, with 48 plants in each row.

In V1, the training system used was 'Alberello' (head-trained spur pruned) (Fig. 1). It had four main branches, each one with one to two spurs, pruned with two to three buds. In V2, the training system was 'Tendone' (overhead trellis, cane pruned) (Fig. 1), with four branches, each with one cane, pruned with four to six buds.

In both vineyards, 10 plants were chosen systematically on an X-shaped path over the whole vineyard (plants numbered 1 through 10), and 10 additional plants were sampled on the two central cross-perpendicular lines (plants numbered 11 through 20). All bunches produced on the east-exposed spur-cane of the 20 plants sampled were collected in each vineyard at ripening. Bunches collected from plants 1 through 10 were numbered progressively based on their position on the spur-cane.

In V1, on the branch chosen, numbers of bunches started from the basal spur and on each spur from the basal bunch. In particular, when one spur with three buds was chosen (Fig. 1), and three shoots with three, two, and two bunches, respectively, developed, bunches were numbered 1 through 3 on the basal shoot, 4 and 5 on the following, and 6 and 7 on the apical shoot. In V2, bunches were numbered starting with the basal shoot of the cane chosen and on each shoot from the basal bunch.

Bunches collected from plants 1 through 10 were weighed and crushed separately; their juices were analyzed separately for OTA content. Bunches collected from plants 11 through 20 were crushed plant by plant, and their juices were analyzed for OTA content.

OTA analysis. In order to determine OTA content, mixture (liquid and solid phase) obtained from crushing each bunch (plants 1 through 10) or bunches of each plant (plants 11 through 20) was weighed, subsampled (25 g), and brought to a volume of 50 ml with an aqueous solution (10 g of polyethylene glycol 8000 and 50 g of sodium bicarbonate dissolved in 1 liter of distilled water). The diluted sample was transferred to a 100-ml plastic centrifuge bottle, mechanically shaken for 30 min, and centrifuged (6,400 $\times$ *g* for 20 min at 0°C); then, 30 ml of the supernatant was filtered (Microfibre filter 1.5 μm, Vicam, Watertown, Mass.). An immunoaffinity column (Ochratest, Vicam) was placed on a solid-phase extraction vacuum manifold (Visiprep, Supelco, Bellefonte, Pa.), and 20 ml of the sample extract was applied to the column, followed by washings with an aqueous solution (5 ml prepared from 25 g of sodium chloride and 5 g of sodium bicarbonate dissolved in 1 liter of distilled water) and distilled water (5 ml). OTA was then slowly eluted from the column with methanol (2 ml) into a glass vial, the eluate was blown dry under nitrogen, and the residue was immediately dissolved in the mobile phase (1 ml) by ultrasonication for a few seconds (Branson Ultrasonic, Danbury, Conn.). The sample was filtered (Cameo 13N, 0.45-μm nylon syringe filter, Micron Separations Inc., Westborough, Mass.) before high-performance liquid chromatography (HPLC) analysis.

The HPLC system consisted of a P-E 200 instrument equipped with an ISS 200 sampling system (Perkin Elmer) and an FP-920 fluorescence detector (Jasco, Tokyo, Japan) set at 333 nm for excitation and 470 nm for emission wavelengths. The system was controlled by Perkin Elmer Turbochrom PC software. A Select B RP-8 column (5-μm particle size, 150 by 4 mm; Merck, Darmstadt, Germany) was employed at ambient temperature, with a mobile phase of acetonitrile–2% acetic acid (41:59 for OTA and 55:45 for OTA methyl ester) at 1.2 ml min^{-1}. The injection volume was 30 μl.

Ochratoxin standard was purchased from Sigma. A solution of OTA (40 mg ml^{-1} in benzene–acetic acid, 99:1, vol/vol) was calibrated spectrophotometrically (Lamda 2, Perkin Elmer) at 333 nm using the value 5,550 liters mol^{-1} cm^{-1} for molar absorptivity *(2)* and stored at −20°C when not in use; after calibration of the OTA solution, working standards were prepared by evaporating an exact volume under a stream of nitrogen and dissolving the residue in the mobile phase.

OTA standards of between 2 and 60 pg were injected into the HPLC. Quantitation was on the basis of peak areas and performed with the Turbochrom PC software. Derivatization of OTA through methylation of the extracts with subsequent HPLC analysis was used for qualitative confirmation of positive samples *(24)*.

The limits of quantitation and detection were determined by the signal-to-noise approach. The analyte response and the chromatographic noise were both measured from the chromatogram of a blank sample extract to which an appropriate amount of OTA had been added. The limits of quantitation (12 ng kg^{-1}) and limits of detection (4 ng kg^{-1}) were defined as those levels resulting in signal-to-noise ratios of approximately 10:1 and 3:1, respectively.

Statistical analysis: OTA content in plants. Mean OTA content for plants 11 through 20 was obtained directly from the chemical analysis, because all the bunches collected from these plants were crushed together to be analyzed for OTA content.

Mean OTA content for plants 1 through 10 was calculated, taking into account the weight of each bunch, as follows:

$$OTA_{plant} = \frac{\sum_{x=1}^{n} (OTA_x \times weight_x)}{\sum_{x=1}^{n} (weight_x)}$$

where n is the number of bunches per plant.

Before statistical analysis was performed, data on OTA were transformed using the natural logarithm (ln) function to make variances homogeneous, because the variance calculated on the original data set was larger than the mean. One was added to the OTA value of each sample before transforming, because the natural logarithm of zero is meaningless. Logarithm transformation allows the shape of data to normalize to an extent that the data may be used in parametric techniques without risk of serious errors *(12).*

A preliminary analysis of these ln-transformed data was performed to verify their distribution, to determine the presence of outliers (using the box plot analysis), and to test them for normality (using the one-sample Kolmogorov-Smirnov goodness-of-fit test). A factorial analysis of variance was also applied to determine the contribution of vineyards, plants within vineyards, and bunches within plants to the total experimental variability. The statistical analyses were performed with SPSS version 11.5.1 (SPSS Inc., Chicago, Ill.).

Afterwards, average OTA content and its 95% confidence interval were calculated over the 20 plants sampled in each vineyard and considered the reference value for the whole vineyard.

Analysis of spatial correlation. Cartesian coordinates were associated with each of the 20 plants sampled in each vineyard based on the row and the position of the plant in the row.

The spatial correlation among OTA contamination of plants in the same vineyard was tested by the semivariance analysis of GS+ (version 3.1.7, Gamma Design Software, Plainwell, Mich.). Semivariance analysis examines the contribution of all pairs of points that are separated by a specific distance, called lag distance, to the total sample variance. The semivariances were then plotted versus the lag distance, fitting them with the best model of semivariogram. This model can be used to estimate the semivariances between the sampled locations (each sampled plant) and any unsampled location (unsampled plants) and to calculate weights of the Kriging function, allowing the estimation of OTA everywhere in the studied area.

Voronoi maps were also drawn using ArcView 8.2 Geostatistical Analyst (ESRI, Redlands, Calif.). These maps are constructed from a series of polygons formed around the location of a sampled point. Voronoi polygons are created so that each location within a polygon is closer to the sampled point in that polygon than any other sampled point. After the polygons are created, neighbors of a sampled point are defined as any other sampled point whose polygon shares a border with the chosen sampled point. According to this definition of neighbors, a local statistic is computed. Polygons are placed in five class intervals by the cluster method; classes range from one to five according to increasing OTA content measured at the sampling point within each polygon. The polygon is colored gray if the class of the polygon is different from each of its neighbors to better distinguish it.

Sampling design. Different methods for sampling bunches were simulated using the data collected from plants 1 through 10 in the two vineyards.

Completely randomized sampling (CRS). Bunches were extracted from the population of bunches produced on plants 1 through 10 at random; samples of $n = 10$ (CRS_10), $n = 20$ (CRS_20), or $n = 40$ (CRS_40) bunches were extracted using the random number generation function available in Excel (Office 2000, Microsoft Corp., Redmond, Wash.).

Systematic sampling: type A (SSA). Bunches were selected from each plant from 1 through 10 in a predefined position; positions ranged from 1, the first bunch of the spur-cane, to 6, the last bunch position present in all the plants. In all these cases, 10 bunches were collected, one bunch per plant ($n = 10$).

Systematic sampling: type B (SSB). The sampled bunches were collected from the central shoot of each spur-cane. In this case, three samples were considered: (i) the basal bunch of the central shoot of the 10 plants ($n = 10$) (SSB_a); (ii) the basal bunch and the next one from the central shoot of the 10 plants ($n = 20$) (SSB_b); and (iii) the basal bunch and the following one from the central shoot of five alternate plants ($n = 10$) (SSB_c), considering either odd or even plants.

Systematic sampling: type C (SSC). Bunches were collected in sequence from a plant starting in position 1 through position 10 ($n = 10$); when the number of bunches in the sampled plant was lower than 10, the fixed number of bunches was completed by collecting bunches from the subsequent plant, starting from position 1.

Mean OTA content and its 95% confidence interval was computed for all the sampling designs, and this was compared to the reference value of the vineyard. The percent difference from a sample and the reference average was computed as follows:

$$\Delta\% = \frac{(OTA_{sample}) - (OTA_{reference})}{(OTA_{reference})} \times 100$$

The t test was applied to verify the significance level of the differences between sample and reference OTA content.

RESULTS

OTA content in bunches. Eighty-one and 71 bunches were collected in V1 and V2, respectively, from plants 1 through 10. In V1, each cane had a minimum of six and a maximum of 14 bunches, while in V2, each cane had between five and nine bunches.

The OTA content of bunches showed high variability, both between bunches within the same plant and from different plants (Table 1). OTA was always detected in bunches collected in V1, with a minimum of 12 and a maximum of 355,886 ng kg^{-1}. In V2, at least one bunch per plant was free from OTA, except in plants 3 and 8, and values higher than 1,000 ng kg^{-1} were rarely detected; only bunch 1 in plant 9 had more than 100,000 ng kg^{-1}.

OTA content per plant was very variable in both vineyards, with a minimum of 356 and 12 ng kg^{-1} and a maximum of 34,330 and 14,564 ng kg^{-1} in V1 and V2, respectively; mean OTA content resulted in 5,531 and 980 ng kg^{-1}, in V1 and V2, respectively.

The analysis of data distribution showed that bunch 1 of plant 9 in V2 is an outlier because it exceeds the interquartile range 1.5 times (Fig. 2); consequently, this bunch was excluded from further analyses. The Kolmogorov-

TABLE 1. *Ochratoxin A content (nanograms per kilogram) in grape bunches and in plants sampled at ripening in San Pancrazio Salentino (Brindisi, South Italy)*[a]

Vineyard 1 (variety 'Negroamaro' head-trained spur pruned)

	Plant no.:									
Position	1	2	3	4	5	6	7	8	9	10
1	54,184	20	2,569	117	387	16	12	236	20	4,709
2	38	37,965	863	189	285	68	2,397	35	28,297	7,684
3	48	55	12	1,725	119	15,880	55	14	4,107	4,105
4	59	39	39,787	229	4,649	573	16	38	187	26,453
5	63	52,830	127	333	227	8,584	38	17	149	104
6	12	8,694	752	640	5,466	1,749	35	99	3,059	606
7	—[b]	—	37,175	—	70	43	82	222	—	90
8	—	—	355,886	—	—	481	22	5,110	—	6,850
9	—	—	859	—	—	43	29	43	—	593
10	—	—	—	—	—	114	—	—	—	—
11	—	—	—	—	—	620	—	—	—	—
12	—	—	—	—	—	27	—	—	—	—
13	—	—	—	—	—	230	—	—	—	—
14	—	—	—	—	—	41	—	—	—	—
Mean	9,136	18,074	34,330	584	1,525	2,594	356	669	4,939	4,250
	Plant no.:									
	11	12	13	14	15	16	17	18	19	20
Mean	3,706	1,867	7,744	1,182	7,412	2,276	478	1,000	1,102	7,044

Vineyard 2 (variety 'Sangiovese' overhead trellis cane pruned)

	Plant no.:									
Position	1	2	3	4	5	6	7	8	9	10
1	0	0	16	0	5	752	0	328	115,735	45
2	3	6	17	52	6	0	24	4	53	12
3	145	229	27	4	0	1,631	38	34	1,617	369
4	98	24	11	0	83	2,673	37	54	155	0
5	45	696	14	28	15	132	1,633	162	0	26
6	—	133	5	272	71	26	20	4	7	13
7	—	52	18	0	3	21	26	128	18	—
8	—	15	—	—	7	—	5	—	—	—
9	—	—	—	—	—	—	55	—	—	—
Mean	71	180	12	47	34	669	271	69	14,564	108
	Plant no.:									
	11	12	13	14	15	16	17	18	19	20
Mean	606	74	384	1.088	317	162	538	177	113	117

[a] In plants 1 through 10 bunches were collected, crushed, and analyzed separately, while in plants 11 through 20 bunches were collected, crushed, and analyzed together per plant.

[b] —, no bunches.

Smirnov test showed that the null hypothesis of correspondence between the distribution of experimental data and the normal distribution cannot be rejected: values of the Z statistic were 0.40 and 0.39, in V1 and V2, respectively, with $P \leq 0.05$. Therefore, data were considered to be normally distributed, averaging 7.88 $\pm$ 1.248 standard deviation (SD) in V1 and 5.04 $\pm$ 1.131 SD in V2.

The analysis of variance showed that only the vineyard had a significant effect on the OTA content of bunches, with V1 more contaminated than V2, and it accounted for 21% of total variability. Plants and bunch positions did not have a significant effect, and they accounted for 7 and 3% of total variability, respectively. The residual 68% was explained by the within bunches variability (data not shown).

Analysis of spatial correlation. The semivariance analysis showed no spatial correlation among OTA content of plants, both in V1 and in V2 (Fig. 3). As a consequence, no models produced a satisfactory fit to a semivariogram, R^2 being lower than 0.2 for all models tested. The absence of a spatial correlation was confirmed by the Voronoi maps (Fig. 4); five polygons colored in gray in V1 and 10 in V2

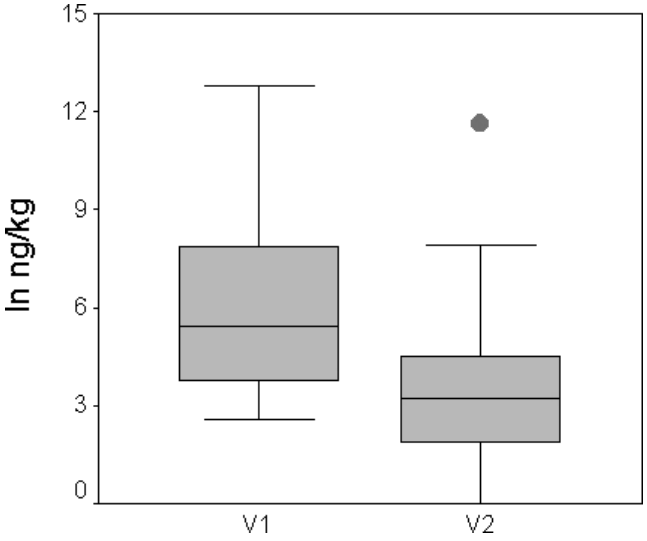

FIGURE 2. *Box and whiskers plot analysis applied to OTA content of all bunches (logarithm transformed) in the two vineyards considered in the study. Box contains 50% of data (interquartile range) and the line inside is the median; whiskers include minimum and maximum values. Points are represented separately only when they exceed 1.5 times the interquartile range.*

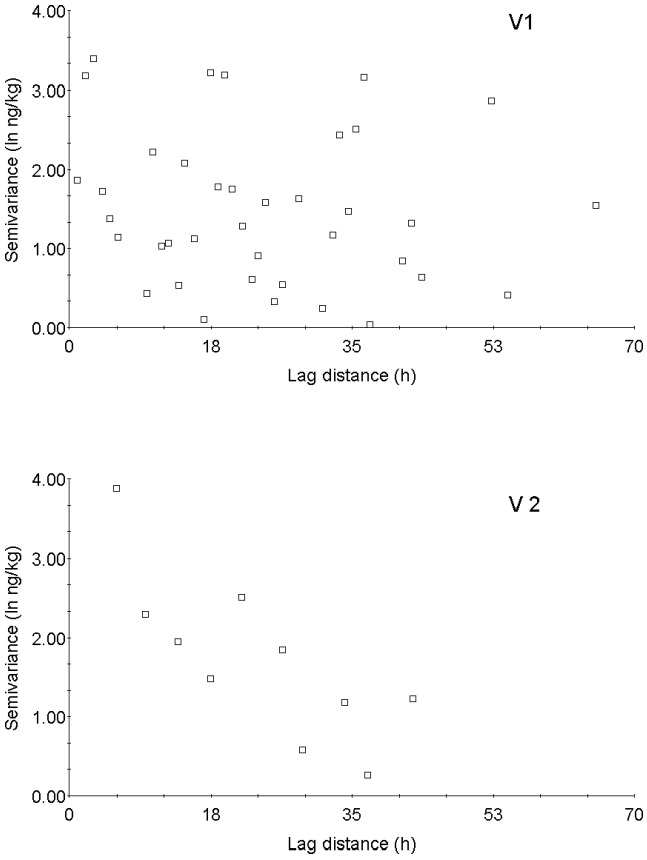

FIGURE 3. *Plotting of semivariances versus lag distance (h), the specific distance that separate all pairs of points, in vineyard 1 (V1) and vineyard 2 (V2).*

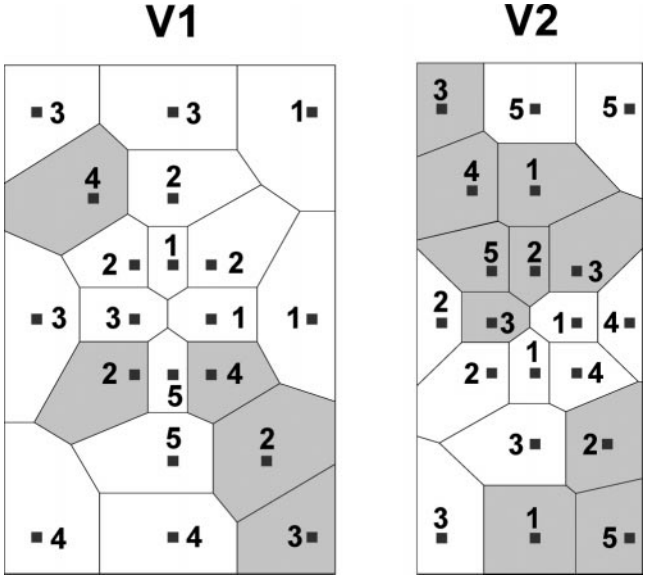

FIGURE 4. *Voronoi maps (see ''Materials and Methods'' for more details) for vineyard 1 (V1) and vineyard 2 (V2) obtained by cluster method. Numbers represent the classes attributed to each polygon based on the value measured at the sampling point (■) within that polygon. The polygon is colored gray if the class of the polygon is different from each of its neighbors to better distinguish it.*

underlined the relevant difference of these sampling points with respect to their neighbors.

Sampling design. Comparison between the reference content of OTA and the different sampling designs is shown in Figure 5. The CRS applied to V1 showed an overestimation of the reference mean, independent of the number of bunches sampled, but the width of the confidence intervals decreased with the number of bunches increasing. In

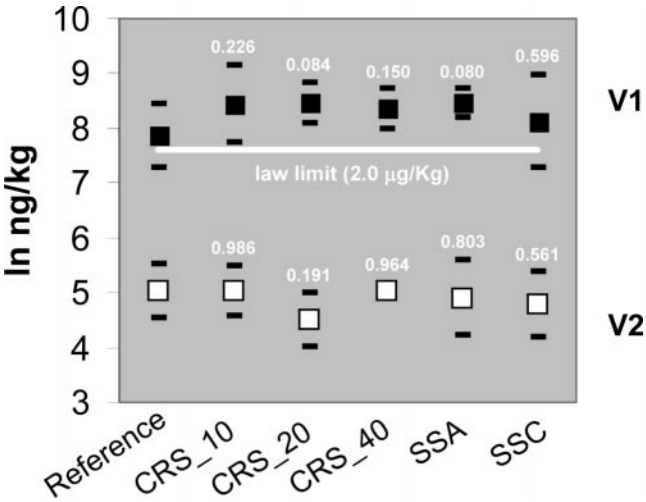

FIGURE 5. *Results of different sampling designs applied to estimate OTA content in vineyards. Mean is represented by the central square for vineyard 1 (V1) (■) and vineyard 2 (V2) (□) and confidence interval by whiskers. Numbers reported represent the probability level of the differences between the reference value and each sample mean, using the* t *test. The white line represents the legal limit of OTA content in must and wine.*

TABLE 2. *Mean ochratoxin A content (ln of nanograms per kilogram data) in bunch samples extracted from the population of bunches in two vineyards at San Pancrazio Salentino (Brindisi, South Italy) using different systematic sampling designs and percent differences with respect to the reference value of the vineyard*

	Vineyard 1		Vineyard 2	
Sampling design[a]	Mean	Δ (%)	Mean	Δ (%)
SSB_a	7.87	0	5.76	10
SSB_b	8.53	8	5.77	10
SSB_c (odd plants)	7.77	−1	5.57	7
SSB_c (even plants)	8.99	14	5.93	13
Reference	7.88	7.30–8.47[b]	5.04	4.54–5.54[b]

[a] SSB_a, one bunch from the central shoot on 10 plants ($n = 10$); SSB_b, two bunches from one or two central shoots on 10 plants ($n = 20$); SSB_c, two bunches from one or two central shoots on five alternate (odd or even) plants ($n = 10$).
[b] 95% confidence interval.

V2, the reference OTA content was better estimated, with the exception of CRS_20, which underestimated the contamination level.

The SSA in V1 produced an estimate of the OTA content very similar to that obtained by sampling with the CRS, but its confidence interval was narrower, especially when the same number of bunches ($n = 10$) was sampled (compare CRS_10 and SSA). Similar results were obtained in V2, but the confidence interval became wider.

The SSC reduced the accuracy of estimates in V1, mostly because the confidence interval was very large, although it was comparable to SSA in V2. The *t* test applied to compare the reference mean with the means obtained by applying any sampling design never produced a significant result, and the sample means were not different from the reference (Fig. 5).

The SSB gave good results, with a difference from the reference value between 0 and 10% when 10 plants were considered. The difference increased when five plants were sampled (Table 2).

DISCUSSION

There are two important factors that can affect sampling variability: the sample selection procedure and the distribution of the toxin in contaminated parts. Generally, with proper sampling techniques, any effect of sample selection can be minimized, but only an increase of sample size can reduce the effects of the uneven distribution of contaminated bunches on sampling variability *(21).* Sampling design is more important than sample size when the object of the study is clustered, while when it is distributed randomly, the number of samples is more relevant *(17).* Distribution of OTA in bunches and in plants is not known; so, in this case, it was necessary to follow an approach that could correctly manage both of these aspects.

In disease surveys of field crops, a common practice is to collect samples at a constant interval along a path of predetermined shape. The sampling design along the X-shaped path of the field, used in this research to choose plants for estimating the mean OTA content of the vineyard, is considered by plant pathologists to be one of the best approaches *(3, 11, 16, 17).*

High variability was observed in OTA content both among bunches and among plants in the two vineyards with different contamination levels. Very high amounts of OTA were detected in one or two strongly contaminated bunches in both vineyards. This is a well-known problem related to mycotoxin quantification reported, for example, for aflatoxin in peanuts *(9),* cottonseed *(10),* and corn *(19).* This variability is totally random; in fact, no relation was found between OTA content and the position of a bunch on the branch or the location of the plant in the vineyard.

Sampling design markedly influenced the assessment of must contamination. The best results were obtained when sampling involved one bunch per plant, in a predefined position, from at least 10 plants. The difference from reference OTA contamination decreased when the number of sampled plants increased. A reduction of variability was also obtained, as expected, increasing the number of sampled bunches.

Despite the large variability among OTA levels detected in bunches, estimated means obtained following different sampling designs did not differ significantly from reference OTA content in both vineyards. If the true contamination in a vineyard is 2 $\mu g\ kg^{-1}$, the limit fixed in Europe for OTA content in must and wine, the range of OTA content assessed using the described sampling designs, should lie between 5 and 0.8 $\mu g\ kg^{-1}$ in the worst situation, when bunches were collected from one to two plants (SSC, $n = 10$) and 2.9 and 1.4 in the best situation, e.g., when the basal bunch of the central shoot was collected (SSB_a, $n = 10$), the position commonly suggested for the evaluation of quality characters at ripening *(6).* The accuracy could be considered acceptable, even if there is a trend towards overestimation.

Quantification of OTA in vineyards makes the correct management of bunches before crushing, with an appropriate timing of harvest and a reduction of time elapsed before crushing in case of high contamination, possible. Then, the wine-making process could be modified accordingly; corrective actions include a controlled time of maceration, the use of selected yeasts for alcoholic fermentation and lactic acid bacteria for malo-lactic fermentation, and the choice of appropriate products for clarification *(5).* Corrective actions determine a variation in the procedures commonly used and increase costs for wine making. The knowledge

of OTA in the vineyard allows lots of bunches with high contamination to be grouped and consequent adoption of these actions. Improved management of the wine production chain can effectively reduce contamination with resulting economic and quality benefits.

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