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Size of uniformity trials for estimating the optimum plot size for vegetables

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ABSTRACT

The aim of this work was to determine the uniformity trial size for estimating the optimum plot size in order to evaluate the fruit mass of tomato, snap-beans and zucchini. The mass of fruits was evaluated in uniformity trials with tomato grown in plastic tunnel in spring-summer and autumn-winter seasons, with snap-beans in plastic greenhouse in autumn-winter season and, with zucchini in plastic greenhouse in summer-autumn and winter-spring seasons. These data were used for planning different sizes of uniformity trials and resampling with replacement was used to estimate the optimum plot size by the method of maximum curvature of the coefficient of variation model. The size of uniformity trials influences the estimation of the optimum plot size for evaluating the mass of fruits of tomato, snap-beans and zucchini. Uniformity trials with tomato with 12 basic experimental units (12 plants) and with snap-beans with 21 basic experimental units (42 plants) are enough for estimating the optimum plot size for evaluating the mass of fruits in plastic tunnel with a confidence interval of 95% minor or equal to two basic experimental units. Uniformity trials with snap-beans with 18 basic experimental units (36 plants) and with zucchini with ten basic experimental units (ten plants) in plastic greenhouse are enough for estimating the optimum plot size for evaluating the mass of fruits with a confidence interval of 95% minor or equal to three basic experimental units.

Keywords: *Solanum lycopersicum*, *Phaseolus vulgaris*, *Cucurbita pepo*, uniformity trials, maximum curvature of the coefficient of variation model.

RESUMO

Tamanho de ensaio de uniformidade para estimação do tamanho de parcela de hortaliças

O objetivo deste trabalho foi determinar o tamanho de ensaio de uniformidade para estimar o tamanho de parcela a fim de avaliar a massa de frutos de tomateiro, de feijão-vagem e de abobrinha italiana. A massa de frutos foi avaliada em ensaios de uniformidade com tomateiro conduzidos em túnel plástico nas estações primavera-verão e outono-inverno, com feijão-vagem em túnel plástico nas estações primavera-verão e outono-inverno, e com abobrinha italiana em estufa plástica nas estações verão-outono e inverno-primavera. Com esses dados foram planejados diferentes tamanhos de ensaios de uniformidade e a reamostragem com reposição foi utilizada para estimar o tamanho de parcela pelo método da curvatura máxima do modelo do coeficiente de variação. O tamanho do ensaio de uniformidade influencia a estimativa do tamanho de parcela para avaliar a massa de frutos de tomateiro, de feijão-vagem e de abobrinha italiana. Ensaios de uniformidade de tomateiro com 12 unidades experimentais básicas (12 plantas) e de feijão-vagem com 21 unidades experimentais básicas (42 plantas) são suficientes para estimar o tamanho de parcela para avaliar a massa de frutos em túnel plástico com amplitude do intervalo de confiança de 95% menor ou igual a duas unidades experimentais básicas. Já ensaios de feijão-vagem com 18 unidades experimentais básicas (36 plantas) e de abobrinha italiana com dez unidades experimentais básicas (dez plantas) são suficientes para estimar o tamanho de parcela para avaliar a massa de frutos em estufa plástica com amplitude do intervalo de confiança de 95% menor ou igual a três unidades experimentais básicas.

Palavras-chave: *Solanum lycopersicum*, *Phaseolus vulgaris*, *Cucurbita pepo*, ensaio de uniformidade, curvatura máxima do modelo do coeficiente de variação.

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Tomato (*Solanum lycopersicum),* snap-bean (*Phaseolus vulgaris*) and zucchini (*Cucurbita pepo*) are crops which stand out in Brazilian horticulture, as they represent significant portion of growing area and production volume. Tomato was the vegetable with the highest production volume in Brazil in 2011 (4.416 million tons), covering a growing area of 71.473 thousand hectares and average productivity of 61.7 t/ha (IBGE, 2011). According to the latest Agricultural Census of Brazil, in 2006, 178,830 thousand tons of zucchini and 56,776 thousand tons of snap-bean were grown (IBGE, 2006).

Due to social and economic importance of tomato, snap-bean and zucchini and aiming to improve production systems, many experiments with these crops are carried out, yearly, in Brazil and all over the world. One of the goals of the researchers during the experiments was to minimize the experimental error in order to obtain accurate inferences about the treatments under evaluation. In this sense, determining the plot size is very important to minimize the experimental error since the experiment planning phase (Storck *et al*., 2011) and maximize information obtained in the available experimental area (Storck *et al*., 2006; Cargnelutti Filho *et al*., 2011b).

Determination of plot size is carried out based on uniformity trials (blank experiments without treatment) and plot size depends directly on the magnitude of the experimental error (Storck *et al*., 2011). The experimental error has its origin in the heterogeneity of the area and experimental material, in application of non-uniform cultural practices, in intraparcelar and interpacelar competition, pests, diseases and weeds occurrence (Storck *et al*., 2011). Moreover, in protected environment, factors such as intensive management and injuries caused by cultural practices and during harvesting (Lúcio *et al*., 2008), proximity of cultivation lines with the sides of plastic tunnels and plastic greenhouses (Lorentz *et al*., 2005), associated with factors inherent to species which have their production evaluated in multiple crops such as tomato, snap-bean and zucchini, are pointed out as inflation factors of experimental error (Lúcio *et al*., 2010b, 2011a).

In order to estimate plot size, several methods are available and, normally, maximum curvature (Lessman & Atkins, 1963) and modified maximum curvature methods (Meier & Lessman, 1971) are used, according to studies carried out by Mello *et al*. (2004), Lúcio *et al*. (2004, 2010a, 2011b, 2012), Lorentz & Lúcio (2009) and Santos *et al*. (2012). According to Paranaiba *et al*. (2009a), the main limitation of these methods is the necessity of grouping the data output of the experimental adjacent basic units, a fact that complicates the use of methods and affect the accuracy of estimating plot size when the uniformity trial is small, as the ones carried out in plastic tunnels and greenhouses, where the size of the trial is commonly restricted to the size of existing facilities (Lorentz & Lúcio, 2009).

In order to overcome these difficulties, Paranaiba *et al*. (2009a) developed the *maximum curvature* of the *coefficient of variation model* (MCCV) to estimate plot size. This method, validated for rice (Paranaiba *et al*., 2009a), wheat and cassava (Paranaiba *et al*., 2009b), corn (Cargnelutti Filho *et al*., 2011a), forage radish (Cargnelutti Filho *et al*., 2011b) and tomato (Lúcio *et al*., 2012), by dispensing grouping data of adjacent basic experimental units, is presented as the most appropriate method to estimate plot size from uniformity trials carried out in areas of restricted size.

Many works had already been carried out aiming to estimate plot size for vegetables grown in restricted areas, as for sweet pepper (Lúcio *et al*., 2004; Lorentz & Lúcio, 2009), zucchini (Mello *et al*., 2004), potato (Oliveira *et al*., 2006), tomato (Lúcio *et al*., 2010a, 2012), lettuce (Lúcio *et al*., 2011b) and snap-bean (Santos *et al*., 2012). However, in none of these works the sizes of uniformity trials used were investigated in order to verify if they are enough to accurately estimate plot size.

Cargnelutti Filho *et al*. (2011b) investigated the influence of the size of uniformity trials to estimate plot size of forage radish green mass. The authors concluded that the size of uniformity trials influences the estimate of plot size, and that trials with 225 basic experimental units (BEU= 0.25 m²) are enough to estimate plot size, for a confidence interval of 95%, minor or equal to one basic experimental unit. Studies on this subject carried out using resampling with replacement methodology can provide useful information to plan experiments with vegetables in restricted areas. Thus, cost reduction avoiding too great uniformity trials is possible and, yet the estimation of plot size through too small-sized and low-reliability uniformity trials is avoided.

Thus, this research aims to determine uniformity trial size to estimate plot size in order to evaluate tomato, snap-bean and zucchini mass.

MATERIAL AND METHODS

Tomato, snap-bean and zucchini fruit mass data were used, collected from uniformity trials carried out in protected environment (plastic tunnel and greenhouse) in the experimental field of Crop Science Department in Federal University of Santa Maria (29°43'S, 53°43'W, altitude 95 m). The local climate is Cfa type, according to Köeppen classification (Moreno, 1961), subtropical and humid, without definite dry season and hot summer. The soil was classified as red haplic acrisol (Streck *et al*., 2008).

The plastic greenhouse used in all trials were Pampeano-arch type, metal structure, 2 m ceiling height and 3.5 m in the center, 20 m length and 10 m width, north-south direction. The plastic tunnel used in all trials owned 3 m ceiling height, 20 m length and 3.6 m width, north-south direction. The protected environments were covered with transparent polyethylene of low density (LDPE), 100 µm thickness.

With salad type tomato in plastic tunnel, two uniformity trials were carried out, one in spring-summer season and other in autumn-winter season, both using hybrid Grandeur. The plants were arranged in three rows of 24 plants each. In two trials, spacing used was 1.2 m between rows and 0.8 m between plants. Each plant was considered one basic experimental unit (BEU) and had its production evaluated separately in each harvest. Ten harvests were carried out in spring-summer season and six harvests in autumn-winter season.

For snap-bean in plastic tunnel in spring-summer and autumn-winter seasons and in plastic greenhouse, in autumn-winter season, cultivar Macarrão was used, totalling three uniformity trials. In plastic tunnel trials, plants were arranged in three rows of 84 plants each. In plastic greenhouse trial, six rows of 72 plants each were arranged. In three trials, spacing used was 1.0 m between rows and 0.2 m between plants. Basic experimental unit (BEU) was consisted of two neighbor plants in the cultivation row, which had its production evaluated jointly in each harvest. Three harvests in spring-summer and four harvests in autumn-winter season were carried out.

For zucchini in plastic greenhouse in summer-autumn and winter-spring seasons, cultivar Caserta was used, totalling two uniformity trials. In both trials, plants were arranged in eight rows of 20 plants each, spacing of 0.9 m between rows and 0.8 m between plants. Each plant was considered one basic experimental unit (BEU) and had their production evaluated separately in each harvest. Twelve harvests were carried out in summer-autumn season and thirty harvests in winter-spring season.

In all uniformity trials, seedlings were transplanted with four true leaves and fertilization and liming were performed according to the results of the soil analyses and recommendations of Brazilian Soil Science Society (Sociedade Brasileira de Ciência do Solo, 2004). Plants were grown on ridges without mulching and drip irrigation system was used. Pests and diseases were controlled preventively, with all cultural management being applied homogeneously, according to the recommendation for the crops.

Total mass of fruits (MF, in grams) was evaluated and planned, in each cultivation row, different uniformity trial sizes due to heterogeneity verified between cultivation rows in experiments in protected environment and recommendation of the use of randomized block design in these cultivation environments (Lorentz *et al*., 2005; Lúcio *et al*., 2006, 2011b). The initial size of uniformity trials (smallest size planned) for all experiments was three adjacent BEUs in row. The final size of uniformity trial (largest size planned) was defined as half the number of BEUs of cultivation row, in order to provide greater number of combinations in resamples. Analyses were carried out considering production of multiple harvests accumulated over time (C1), (C1+C2), (C1+C2+...+Cn), in which "n" shows the last harvest done.

For each uniformity trial sizes planned, 3,000 resamples with reposition and estimation was carried out, for each sample, of the spatial autocorrelation coefficient of the first order (p), variance (s^2) and mean (m), which were used to calculate optimum plot size (Xo) using the expression $Xo = \frac{10\sqrt[3]{2(1-p^2)s^2m}}{m}$ (Paranaiba *et al*., 2009a). Percentile values 2.5, mean and percentile 97.5 of Xo were also estimated.

For resamples, adjacent BEUs in cultivation row were selected, in order to form different sizes of uniformity trials. To illustrate these resamples, the matrix of the first row of tomato uniformity trial was taken as basis in tunnel in spring-summer (24 BEUs). In order to determine the first sample of initial size of uniformity trials (three BEUs), one BEU between the first and the 22nd BEU on the cultivation row was selected. Assuming that the first BEU was selected, so the first uniformity trial would consist of the first, second and third BEU. For the second sample, which was also randomly selected, one BEU between the first and 22nd BEU on cultivation row was selected. Assuming that the 22nd BEU has been selected, so the second uniformity trial would be consisted of 22nd, 23rd and 24th BEU. The same procedure was carried out for the remaining 2,998 trial samples with three BEUs.

For size of uniformity trial of four adjacent BEU in cultivation row, the following procedure was carried out: for first sample, which was also randomly selected, one BEU between the first and the 21st BEU was selected on the cultivation row. Assuming that the 21st BEU has been selected, so the first uniformity trial would be consisted of 21st, 22nd, 23rd and 24th BEU. The same procedure was carried out for the remaining 2,999 trial samples with four BEUs. For the other uniformity trial sizes planned, the same procedure of the trials with three and four BEUs were carried out.

For statistics Xo, width of confidence interval of 95% (CI 95%), by the difference between percentiles 97.5 and 2.5 was calculated. Afterwards, size of uniformity trial was determined, in BEUs, to estimate plot size, from initial size (three BEUs) and considering as size of uniformity trial the number of BEUs from width of confidence interval of 95% of Xo was minor or equal to two BEUs. Statistical analyses were performed using computer program R (R Development Core Team, 2014) and Office Excel application.

RESULTS AND DISCUSSION

The mean of plot size, as well as, percentiles 2.5 and 97.5 of plot size (Xo), obtained from 3,000 resamples carried out for each uniformity trial sizes planned for tomato, snap-bean and zucchini showed behavior difficult to interpret and with no biological meaning when they were used in analyses of fruit mass of the first harvest (C1) and accumulated production data up to harvest which represent 50% of total harvest carried out in each experiment.

This behavior possibly results from high variability which can be noticed between fruit mass obtained in initial harvests even between neighbor plants, due to factors such as differences in seedling vigor and necessity to replant plants (Lorentz *et al*., 2005). During production cycle, through the accumulation of fruit mass data obtained from plants in successive harvests carried out, these oscillations tend to be smoothed, or even to disappear (Lúcio *et al*., 2008, 2010a, 2011b), which results in better results adjusted to the analysis model, easy to interpret and with biological significance. In this sense, only results from the analyses carried out with total fruit mass obtained per BEU are presented and discussed (Figure 1, Table 1).

Generally, regardless the crop which was evaluated (tomato, snap-bean or zucchini), growing environment (plastic tunnel or greenhouse), season or cultivation row, with an increase of uniformity trial sizes planned, gradual increase in the estimated average plot size occurred (Xo) (Figure 1). Cargnelutti Filho *et al*. (2011b) observed this same behavior for mean of plot size of forage radish green mass. This result shows that when plot size (Xo) is estimated from small uniformity trials, a subestimation of plot size tends to occur. On further experiments with the crop, using subestimate plot sizes compromises the reliability of experiment results, because in this situation an increase of experimental error occurs (Storck *et al*., 2011). This fact reinforces the importance to determine uniformity trial size to estimate more precisely the plot size to be used in experiments with the studied crops.

Width of confidence interval of 95%

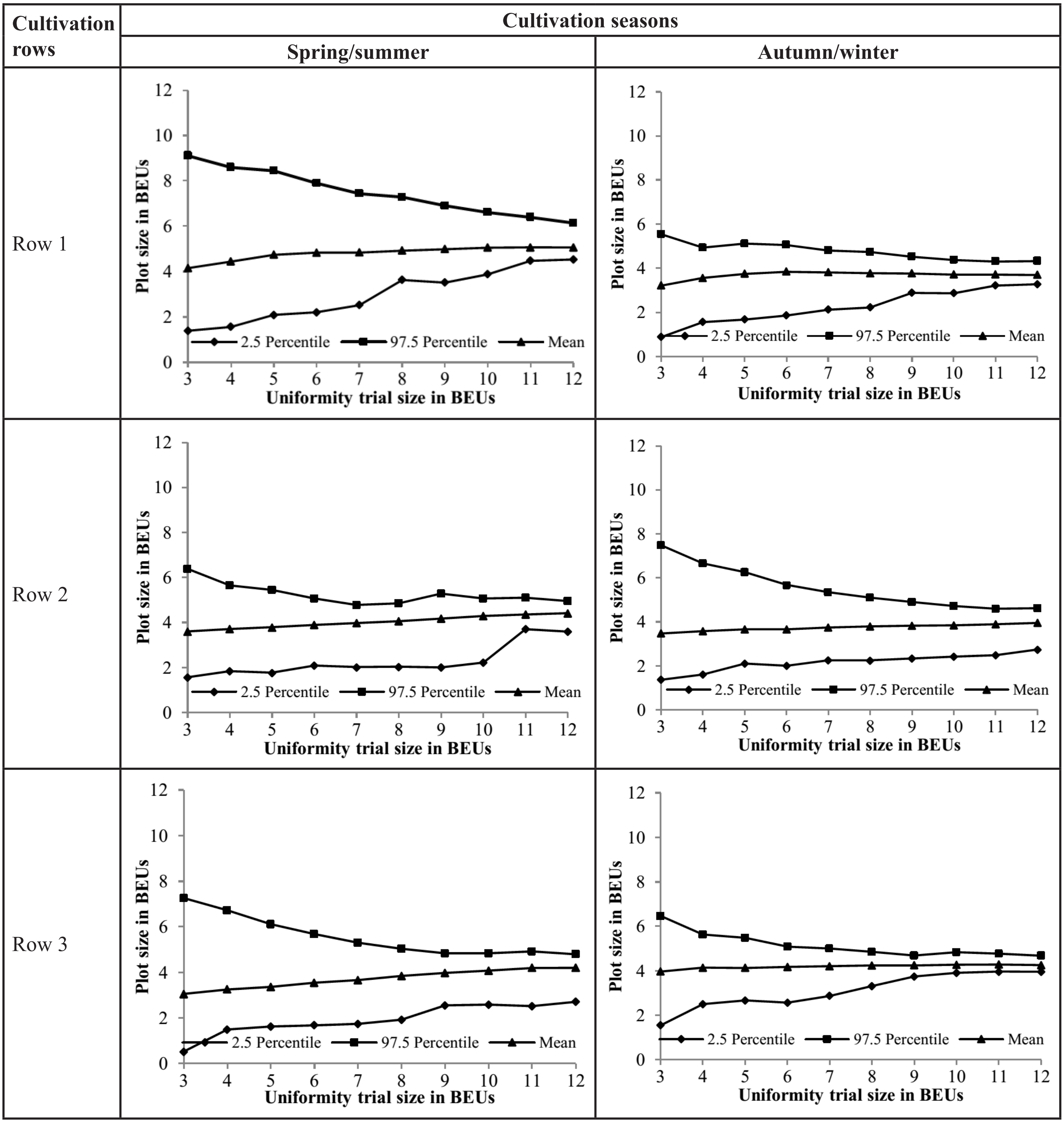

Figure 1. Graphical representation for statistics percentile 2,5, mean and percentile 97,5 of 3.000 estimates of optimum plot size (Xo) for total mass of fruits of tomato in plastic tunnel in spring-summer and autumn-winter seasons, in basic experimental units (BEUs), for different uniformity trials size {representação gráfica das estatísticas percentil 2,5, média, percentil 97,5 das 3.000 estimativas do tamanho de parcela (Xo) para massa total de frutos de tomateiro em túnel plástico nas estações de cultivo primavera-verão e outono-inverno, em unidades experimentais básicas (BEUs), para os diferentes tamanhos de ensaios de uniformidade}. Santa Maria, UFSM, 2014.

(CI 95%) of plot size (Xo), calculated from percentiles 97.5 and 2.5, showed decreasing behavior in all cultivation rows, regardless of crop, season and growing environment, as greater uniformity trial sizes were planned (Figure 1). Taking as an example the first row of the experiment with tomatoes in plastic tunnel in spring-summer (Figure 1), width of 95% on confidence interval for Xo in 3,000 resamples of uniformity trials with dimensions of three BEUs was 7.7 BEUs, whereas for the trial with dimension of 12 BEUs was 1.6 BEUs. Thus, the authors concluded that estimations of plot sizes carried out from small uniformity trials are little precise, due to present great width between sizes obtained. Cargnelutti Filho *et al.* (2011b) also concluded that small uniformity trials result in not very

Table 1. Uniformity trials size (BEU) to estimate the optimun plot size in order to evaluate the mass of fruits of tomato, snap-beans and zucchini with a confidence interval of 95% (CI 95%) minor or equal to two or three basic experimental units (BEU) {tamanho de ensaio de uniformidade (BEU) para estimar o tamanho de parcela para avaliar a massa de frutos total de tomateiro, feijão-vagem e abobrinha italiana com amplitude do intervalo de confiança de 95% (CI 95%) menor ou igual a duas ou três unidades experimentais básicas (BEU)}. Santa Maria, UFSM, 2014.

Crop	Environment	Season	CI 95% ≤ 2	CI 95% ≤ 3
Tomato	tunnel	P/V	11	10
Tomato	tunnel	O/I	12	8
Snap-bean	tunnel	P/V	21	12
Snap-bean	tunnel	O/I	16	12
Snap-bean	greenhouse	O/I	-	18
Zucchini	greenhouse	V/O	-	10
Zucchini	greenhouse	I/P	-	9

P/V= spring/summer (primavera/verão); O/I= autumn/winter (outono/inverno); V/O= summer/autumn (verão/outono); I/P= winter/spring (inverno/primavera); -= condition which estimating the optimun plot size with established accuracy was not possible (situação onde não foi possível estimar o tamanho de parcela com a precisão estabelecida).

reliable plot size estimates for forage radish green mass.

Size of uniformity trials to evaluate tomato, snap-bean and zucchini fruit mass influences plot size and their estimate accuracy, situation also verified by Cargnelutti Filho *et al*. (2011b) for forage radish. On the other hand, Storck *et al*. (2006) concluded that size of uniformity trials to evaluate potato tuber productivity does not influence the estimate of plot size, result which can be attributed to the fact that the smallest uniformity trial sizes planned, on that study, was high (288 BEUs), that means, this size was larger than the minimum necessary, resulting in unnecessary costs and efforts in order to conduct the uniformity trial. These results highlight the importance to determine uniformity trial sizes which gives the researcher the accuracy wanted to estimate plot size (Xo).

The results presented in Figure 1 and in Table 1 should be used by the researchers as support to choose the number of BEUs of their uniformity trials, taking into account the level of accuracy desired for estimating plot size (Xo). The judgment of the maximum acceptable width of CI 95% to estimate optimum plot size from uniformity trials is not one aim on this study, in this case, the researcher will be the one to decide for this definition when using this information for experimental planning.

Considering an only recommendation for plot size in order to evaluate tomato fruit mass grown in plastic tunnel, regardless cultivation row and season, uniformity trials with 12 BEUs (12 plants) are enough to estimate plot size with width of confidence interval of 95% minor or equal to two BEUs (Figure 1, Table 1). Estimates with this level of precision seem appropriate for planning experiments in restricted environments and, so, uniformity trials with these dimensions are enough to estimate plot size. Actually, nowadays uniformity trials with 24 BEUs in plastic tunnels are used (Lúcio *et al*., 2010a, 2012) and greenhouses (Lopes *et al*., 1998; Lúcio *et al*., 2010a) to estimate tomato plot size, which confirms the importance and the practical validity of the results found in this work.

For snap-bean in plastic tunnel, regardless of cultivation row, in order to obtain one estimate of plot size with width of confidence interval of 95% for Xo minor or equal to two BEUs (four plants) uniformity trials with 21 BEUs (42 plants) are necessary in spring-summer season and with 18 BEUs (36 plants) in autumn-winter season (Table 1). On the other hand, in plastic greenhouse in autumn-winter, uniformity trials with the same 18 BEUs (36 plants) allow to estimate plot size (Xo) with width of CI 95% minor or equal to three BEUs (Table 1). Santos *et al*. (2012) used uniformity trials with 42 BEUs (84 plants) in plastic tunnel and with 36 BEUs (72 plants) in plastic greenhouse to estimate plot size for snap-bean.

Uniformity trials with ten BEUs (ten plants) in plastic greenhouse in winter-spring and summer-autumn seasons are enough to estimate plot size (Xo) of zucchini fruit mass with width of CI 95% minor or equal to three BEUs (three plants) (Table 1). Mello *et al*. (2004), working with zucchini used uniformity trials with 20 BEUs (20 plants) to estimate plot size. With snap-bean and zucchini in plastic greenhouse, new studies can be carried out in order to determine uniformity trial size sufficient for a more precise estimate of plot size. In these studies, original uniformity trials should have a higher number of BEUs per cultivation row, in a way that they can be planned in resamples uniformity trials with more than 18 BEUs (36 plants) with snap-bean and with more than ten BEUs (ten plants) with zucchini.

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