Revista Ciência Agronômica, v. 46, n. 3, p. 597-606, jul-set, 2015
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Artigo Científico
ISSN 1806-6690

Uniformity trial size in estimates of plot size in restrict areas[1]

Tamanho de ensaio de uniformidade para estimação do tamanho de parcela em áreas restritas

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ABSTRACT - The aim of this study was to determine the uniformity trial size when estimating optimum plot size in order to evaluate fresh phytomass in lettuce plants and fruit weight in sweet peppers. Production data, collected in uniformity trial on lettuce in a plastic greenhouse in both summer and winter, lettuce in plastic tunnels in autumn and winter, and sweet pepper in a plastic greenhouse in the summer-autumn and spring-summer seasons, were used to plan different uniformity trial sizes in crop rows. In all the experiments, each plant was evaluated individually and considered as a basic experimental unit. For each size in a uniformity trial, 3,000 resamples, randomly taken with replacement, were used to estimate optimum plot size. Uniformity trial using 27 basic experimental units to evaluate the fresh phytomass of lettuce plants, and with 29 basic experimental units to assess fruit weight in sweet pepper, are sufficient to estimate optimum plot size, with an amplitude of the 95% confidence interval of less than or equal to two basic experimental units.

Key words: *Lactuca sativa*. *Capsicum annuum*. Experimental design.

RESUMO - O objetivo deste trabalho foi determinar o tamanho de ensaio de uniformidade para estimar o tamanho ótimo de parcela a fim de avaliar a fitomassa fresca de plantas de alface e a massa de frutos de pimentão. Dados de produção coletados em ensaios de uniformidade com alface em estufa plástica nas estações verão e inverno, com alface em túnel plástico nas estações outono e inverno, e com pimentão em estufa plástica nas estações verão-outono e primavera-verão, foram utilizados para planejar diferentes tamanhos de ensaios de uniformidade dentro das linhas de cultivo. Em todos os experimentos cada planta foi avaliada, individualmente, e considerada uma unidade experimental básica. Para cada tamanho de ensaio de uniformidade, foram realizadas aleatoriamente, e com reposição, 3.000 reamostras, que foram utilizadas para estimar o tamanho ótimo de parcela. Ensaios de uniformidade com 27 unidades experimentais básicas para avaliar a fitomassa fresca de plantas de alface e com 29 unidades experimentais básicas para avaliar a massa de frutos de pimentão são suficientes para estimar o tamanho ótimo de parcela com amplitude do intervalo de confiança de 95% menor ou igual a duas unidades experimentais básicas.

Palavras-chave: *Lactuca sativa*. *Capsicum annuum*. Planejamento experimental.

DOI: 10.5935/1806-6690.20150043
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[1]Recebido para publicação em 19/02/2014; aprovado em 31/03/2015
Parte da Tese de Doutorado do primeiro autor apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal de Santa Maria/UFSM
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INTRODUCTION

The lettuce (*Lactuca sativa*) and sweet pepper (*Capsicum annuum*) are important crops for Brazilian horticulture. According to the most recent Brazilian Agricultural Census, in 2006 the lettuce was the leaf vegetable with the largest production volume (525,602,000 tonnes), while production of the sweet pepper was 276,767,000 tonnes (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2006).

Because of the socio-economic importance of lettuce and sweet peppers, and the need to improve cultivation techniques, every year several experiments are carried out on these crops, with an aim to minimising experimental error and obtaining accurate conclusions about the treatments under evaluation.

When cultivating vegetables in a protected environment, variability is introduced due to the proximity of the crop rows to the sides of the plastic tunnels (LORENTZ *et al*., 2005), to intensive management and damage to the crop (LÚCIO *et al*., 2008), to faults in the irrigation system (CARPES *et al*., 2008), to leaks and infiltration, to shading by the structure and to the heterogeneity of the soil (LÚCIO *et al*., 2011). To this effect, designing experiments in tunnels and plastic greenhouses by determining plot size is essential for increasing experimental precision (STORCK *et al*., 2011) and maximising the information obtained from the available experimental area (CARGNELUTTI FILHO *et al*., 2011a; STORCK; BISOGNIN; OLIVEIRA, 2006).

To estimate plot size, uniformity trial are carried out, also known as blank trials (with no treatment), in which the whole experimental area is managed uniformly, and the individual production data of each plant is evaluated in order to learn the variability of the area and estimate plot size (STORCK *et al*., 2011).

Paranaiba, Ferreira e Morais (2009) developed the method of maximum curvature of the coefficient of variation model (MCCV) for estimating plot size from uniformity trial. This method has been validated for rice (PARANAIBA; FERREIRA; MORAIS, 2009), wheat and manihot (PARANAIBA; MORAIS; FERREIRA, 2009), maize (CARGNELUTTI FILHO *et al*., 2011b), wild radish (CARGNELUTTI FILHO *et al*., 2011a) and tomato (LÚCIO *et al*., 2012). According to Paranaiba, Ferreira e Morais (2009), the advantage of the MCCV method over the methods of maximum curvature (LESSMAN; ATKINS, 1963) and modified maximum curvature (MEIER; LESSMAN, 1971) is that the grouping of data from adjacent basic experimental units is not necessary. This suggests that the MCCV method is the most suitable for estimating plot size from small uniformity trial carried out in tunnels and plastic greenhouses, where the trial is generally restricted to the size of the existing facilities (LORENTZ; LÚCIO, 2009).

Some studies have been carried out to estimate plot size in the sweet pepper (LORENTZ; LÚCIO, 2009; LÚCIO *et al*., 2004), tomato (LÚCIO *et al*., 2010, 2012.), lettuce (LÚCIO *et al*., 2011) and green bean (SANTOS *et al*., 2012). However, these studies were not concerned with investigating whether the sizes used in the uniformity trial were sufficient to accurately estimate the plot size.

Cargnelutti Filho *et al.* (2011a) concluded that, in the wild radish, the size of the uniformity trial has an influence on estimates of plot size, and that trials with 225 basic experimental units (BEU) are sufficient to estimate plot size for an amplitude of the 95% confidence interval of less than or equal to one BEU.

The MCCV method was used by Lúcio *et al.* (2012) to estimate plot size in the tomato, but there is no information on the influence of the size of the uniformity trial on the reliability of estimates of plot size in vegetables. The aim of this work therefore was to determine the uniformity trial size, so as to estimate plot size in restrict áreas.

MATERIAL AND METHODS

Data on the fresh phytomass from lettuce plants and the fruit weight of sweet pepper plants were used, collected in uniformity trial (experiments without the application of treatments), which were carried out in a protected environment (tunnel and plastic greenhouse) in the experimental area of the Department of Plant Science of the Federal University of Santa Maria (UFSM) (latitude 29°43'S, longitude 53°43'W, at an altitude of 95m), in Santa Maria, in the state of Rio Grande do Sul, Brazil (Table 1). The climate in the region according to the Köppen classification (MORENO, 1961) is of type Cfa (humid subtropical, no defined dry season, with hot summers). The soil is classified as an arenic dystrophic Red Argisol (STRECK *et al*., 2008).

The plastic greenhouse used in the experiments has a metal quonset-type structure, a ceiling height of 2 m with 3.5 m in the centre, a length of 20 m and a width of 10 m, and is oriented in a north-south direction. The plastic tunnels have a ceiling height of 3 m, are 20 m long, 3.6 m wide and oriented north to south. The covering for these protected environments was of low density polyethylene plastic film with a thickness of 100 microns.

Table 1 - Summary of the uniformity trial carried out with the lettuce and sweet pepper crops and a breakdown of the planned sizes of the uniformity trial within each crop row for the estimation of the optimum plot size

Crop	Environment	Season	Nº of rows	Nº of plants per row	Nº of BEUs(1)	Uniformity trial planned in BEUs
Lettuce	Greenhouse	Winter	6	48	288	3, 4, ..., 23, 24
Lettuce	Greenhouse	Summer	6	48	288	3, 4, ..., 23, 24
Lettuce	Tunnel	Autumn	3	64	192	3, 4, ..., 31, 32
Lettuce	Tunnel	Winter	3	60	180	3, 4, ..., 29, 30
Pepper	Greenhouse	Summer-Autumn	8	70	560	3, 4, ..., 34, 35
Pepper	Greenhouse	Spring-Summer	8	70	560	3, 4, ..., 34, 35

(1)BEUs, basic experimental unit of one plant

The lettuce cultivar Vera was used for the crop grown in a plastic greenhouse during the summer and winter, and the cultivar Amanda for the crop grown in plastic tunnels during the autumn and winter, giving a total of four experiments. In the experiments in the greenhouse, the plants were arranged in six rows of 48 plants each. In the experiment in the tunnels, the plants were arranged in three rows of 64 plants each in the autumn, and in three rows of 60 plants each in the winter (Table 1). In the four experiments the rows were spaced one metre apart with 0.3 m between plants.

For the crop of sweet pepper, the Vidi cultivar was used, grown in a plastic greenhouse in the summer-autumn and spring-summer seasons. In the two experiments, the crop was grown in eight rows of 70 plants each, with one meter between rows and 0.30 m between plants (Table 1).

In the four experiments on the lettuce and the two on the sweet pepper, the seedlings were transplanted when showing four true leaves, and fertilisation and liming carried out according to the results of the soil analysis and the recommendations of the Sociedade Brasileira de Ciência do Solo (2004). The plants were grown on ridges with no mulching, and irrigation was by drip. Pests and diseases were controlled preventively, with all the managements being applied uniformly, as per the recommendations for each crop.

In all the experiments each plant was evaluated individually and considered as a basic experimental unit (BEU). In the experiments with the lettuce, the fresh phytomass (FP in grams) was evaluated, and with the sweet pepper, the weight of the fruit (FW in grams) was taken.

Different sizes for the uniformity trial were planned within each crop row (Table 1), based on the FP data for the lettuce and the FW for the sweet pepper, and due to the heterogeneity seen between rows in the experiments in the protected environment (LORENTZ *et al*., 2005; LÚCIO *et al*., 2006, 2011). The initial sample size (the smallest) in the uniformity trial in all the experiments was three adjacent BEUs in any one row. The final size (the largest) was set as half the number of BEUs per row (Table 1).

Three thousand resamples with replacement were carried out for each of the planned sizes for the uniformity trial, and for each resampling the first-order spatial autocorrelation coefficient (p), variance (s^2), mean (m) and optimum plot size (Xo) were estimated using expression 1:

$$Xo = \frac{10\sqrt[3]{2(1-p^2)S^2m}}{m} \quad (1)$$

the coefficient of variation at the optimum plot size (CV) using expression 2:

$$CV = \frac{\sqrt{(1-p^2)S^2/m^2}}{\sqrt{Xo}} x100 \quad (2)$$

(PARANAIBA; FERREIRA; MORAIS, 2009), and the coefficient of variation of the uniformity trial (CVe). The minimum, 2.5 percentile, mean, 97.5 percentile and maximum values of p, s^2, m, Xo, CV and CVe were also estimated.

When resampling, adjacent BEUs in a crop row were selected to make up the different sizes in the uniformity trial. As an example of this resampling, the matrix of the first row in the trial on the summer lettuce from the greenhouse was used as a base (48 BEUs). To establish the first resample for the initial sample size of the trial (three BEUs), one BEU between the first and the 46 th BEU in the crop row was selected randomly. Assuming that the first BEU was selected, then the first uniformity trial would comprise the first, second and third BEUs. For the second resampling, one BEU between the first and the 46 th BEU in the crop row was again selected randomly. Assuming that the 46 th BEU was selected, then the uniformity trial would comprise the 46 th, 47 th and 48 th BEUs. The same procedure was followed for the remaining 2,998 resamples in the trials with three BEUs.

For sample size in the uniformity trial using four adjacent BEUs in the crop row, the following procedure was employed: in the first resampling, one BEU between the first and the 45 th BEU in the row was selected at random. Assuming that the 45 th BEU was selected, then the first uniformity trial would comprise the 45 th, 46 th, 47 th and 48 th BEUs. The same procedure was followed for the remaining 2,999 resamples in the trials with four BEUs. For the remaining planned sample sizes in the uniformity trial (Table 1), the same procedure was followed as in the trials using three and four BEUs.

For the Xo statistic, the amplitude of the 95% confidence interval was calculated from the difference between the 2.5 and 97.5 percentiles. Subsequently the uniformity trial size in BEUs was determined for estimating the optimum plot size, starting with the initial size (three BEUs) and taking as the trial size the number of BEUs at which the amplitude of the 95% confidence interval was less than or equal to two BEUs. Statistical analyses were performed using the R software (R DEVELOPMENT CORE TEAM, 2014).

RESULTS AND DISCUSSION

The fresh phytomass (FP) of the winter lettuce evaluated in the greenhouse varied from 55 to 229 g plant^{-1}, the average being 124.01 g plant^{-1} with a coefficient of variation (CV) of 31.88%. Also in the greenhouse, but in the summer, the FP of the lettuce varied from 100 to 474 g plant^{-1} with an average of 240.72 g plant^{-1} and a CV of 32.64%. The FF of the lettuce cultivated under tunnels in the winter ranged from 50 to 236 g plant^{-1}, the average being 130.27 g plant^{-1} with a CV of 36.16%. For the lettuce under tunnels, but in the autumn, the FF ranged from 117 to 433 g plant^{-1}, the average being 290.18 g plant^{-1} with a CV of 24.07%. The fruit weight (FW) of the sweet pepper evaluated in the greenhouse in the summer-autumn varied from 140 to 1681 g plant^{-1}, with an average of 751 g plant^{-1} and a CV of 37.04%. For the sweet pepper in the greenhouse, but in the spring-summer, the FW varied from 284 to 2916 g plant^{-1}, with an average of 1,186 g plant^{-1} and a CV of 36.53%. Consequently, the various uniformity trial on the lettuce and sweet pepper are seen to lend credibility to the proposed study, as they display levels of productivity which reflect the actual conditions of experiments conducted in protected environments and, above all, a variability that is important in the study of the optimum plot size and of the uniformity trial.

Independent of the crop (lettuce or sweet pepper), environment (tunnel or greenhouse), season or crop row, the mean estimate of the optimum plot size (Xo) showed a gradual increase with the increase in size of the uniformity trial (Figures 1, 2, 3 and 4). This same behaviour was seen in the fresh weight of the wild radish by Cargnelutti Filho *et al.* (2011a). The result suggests that estimates of optimum plot size carried out with uniformity trial having few BEUs in the crop row underestimate the optimum plot size, which in turn increases experimental error and may consequently reduce the reliability of the results of future experiments (STORCK *et al*., 2011).

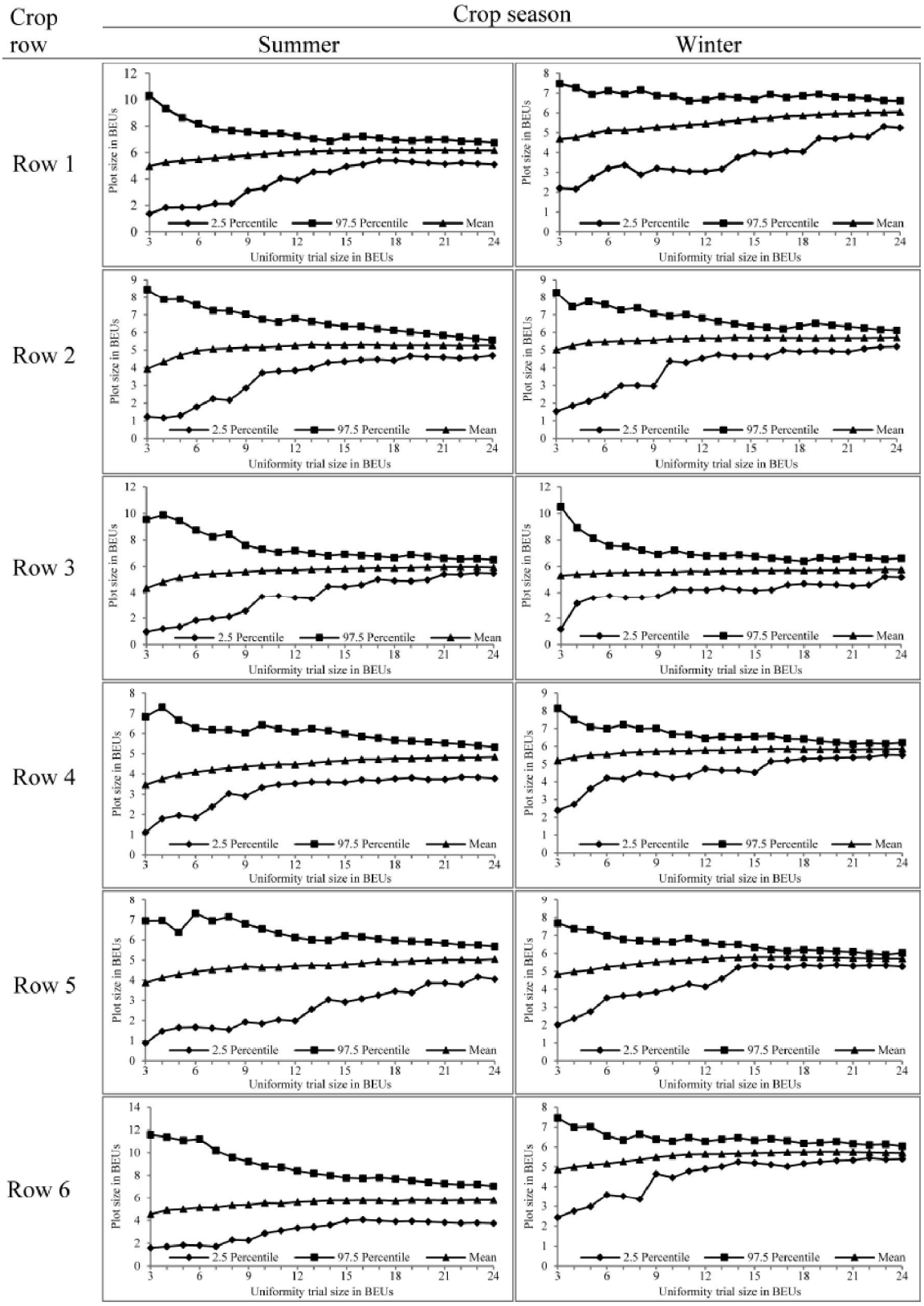
With the increase in size of the uniformity trial, it was found for both crops, that for all seasons and crop rows there was a reduction in the amplitude of the 95% confidence interval (CI 95%) for optimum plot size (Xo) (Figures 1, 2, 3 and 4). Taking as an example the first row in the experiment with sweet pepper for the summer-autumn (Figure 3), the amplitude of CI 95% for Xo in the 3,000 resamples of the uniformity trial at a size of three BEUs was 6.6 BEUs, while for the trial at a size of 35 BEUs, it was 0.4. It can therefore be inferred that estimates for Xo made from trials with few BEUs in the crop row have high variability and therefore low reliability. Cargnelutti Filho *et al*. (2011a) also concluded that small uniformity trial when evaluating fresh weight in the wild radish give estimates for optimum plot size of broad variability and low reliability.

The results show that the uniformity trial size when evaluating fresh weight in lettuce and fruit weight in sweet peppers, measured in BEUs in the crop row, influences the optimum plot size and the reliability of the estimate; a condition also seen by Cargnelutti Filho *et al*. (2011a) in the wild radish. On the other hand, Storck, Bisognin e Oliveira (2006) concluded that the uniformity trial size in the potato does not affect estimates of optimum plot size. Cargnelutti Filho *et al.* (2011a) inferred that this result could be attributed to the smallest planned size for the uniformity trial being high (288 BEUs) and at a level which was greater than the required minimum, resulting in unnecessary cost and effort when conducting the uniformity trial, and thus highlighting the importance of determining a size for the trial in BEUs which would give the researcher the desired accuracy for estimating the optimum plot size (Xo).

Both the mean and amplitude for CI 95% of the optimum plot size (Xo), as a function of the size of the uniformity trial, varied between the lettuce and sweet pepper crops, between crop rows in the same experiment, between seasons for the same row and crop, and between growing environments for the same crop, row and time of year (Figures 1, 2, 3 and 4).

In particular, the variability in estimates for Xo between the rows of any one trial confirms the hypothesis that it is more prudent to carry out studies into the size

Figure 1 - Graphical representation of 2.5 percentile, mean and 97.5 percentile statistics for the 3,000 estimates of optimum plot size (Xo) for the fresh phytomass of lettuce in a plastic greenhouse, in basic experimental units (BEUs), for different sizes of the uniformity trial in BEUs



of the uniformity trial and the plot size in vegetables grown in restrict areas considering each crop row as a blank trial, as recommended by Lorentz *et al.* (2005) and Lúcio *et al*. (2006) in the sweet pepper, Lúcio *et al.* (2011) in the lettuce, Santos *et al.* (2012) in the green bean, and Lúcio *et al.* (2012) in the tomato, in view of the heterogeneity observed between crop rows due to the border effect, which acts differently for plants at the sides when compared to those in the centre of plastic tunnels and greenhouses (LORENTZ *et al*., 2005).

Each researcher will be able to choose a determined number of BEUs in their uniformity trial, taking into account the level of precision required in estimating the optimum plot size (Xo) seen in Figures 1, 2, 3 and 4. Here is not the place to judge the maximum acceptable amplitude of CI 95% for estimating the optimum plot size from uniformity trial, this being left to the criteria of any researcher who takes advantage of this information in planning their experiments.

For lettuce, considering a single recommendation regardless of the crop row, uniformity trial of 22 BEUs are needed in the autumn and 27 BEUs in the winter for crops in plastic tunnels, and of 20 BEUs in the summer (with the exception of row six, which did not reach an amplitude for CI 95% of less than three BEUs) and 23 BEUs in the winter for crops in the greenhouse, to

Figure 2 - Graphical representation of 2.5 percentile, mean and 97.5 percentile statistics for the 3,000 estimates of optimum plot size (Xo) for fresh phytomass of lettuce grown in plastic tunnels, in basic experimental units (BEUs), for different sizes of the uniformity trial in BEUs

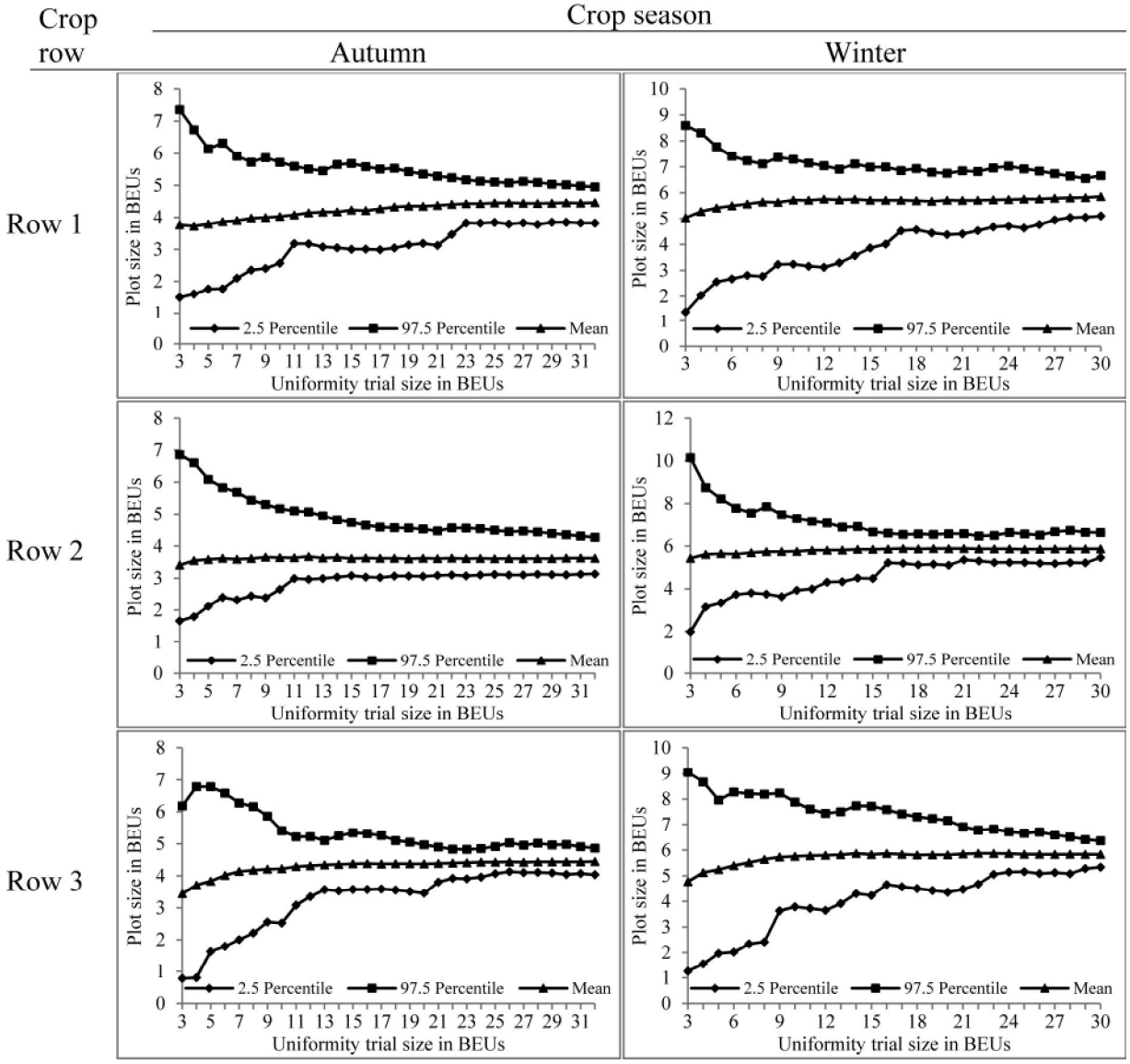


Figure 3 - Graphical representation of 2.5 percentile, mean and 97.5 percentile statistics for the 3,000 estimates of optimum plot size (Xo) for fruit weight of sweet pepper grown in a plastic greenhouse, in basic experimental units (BEUs), for different sizes of the uniformity trial in BEUs

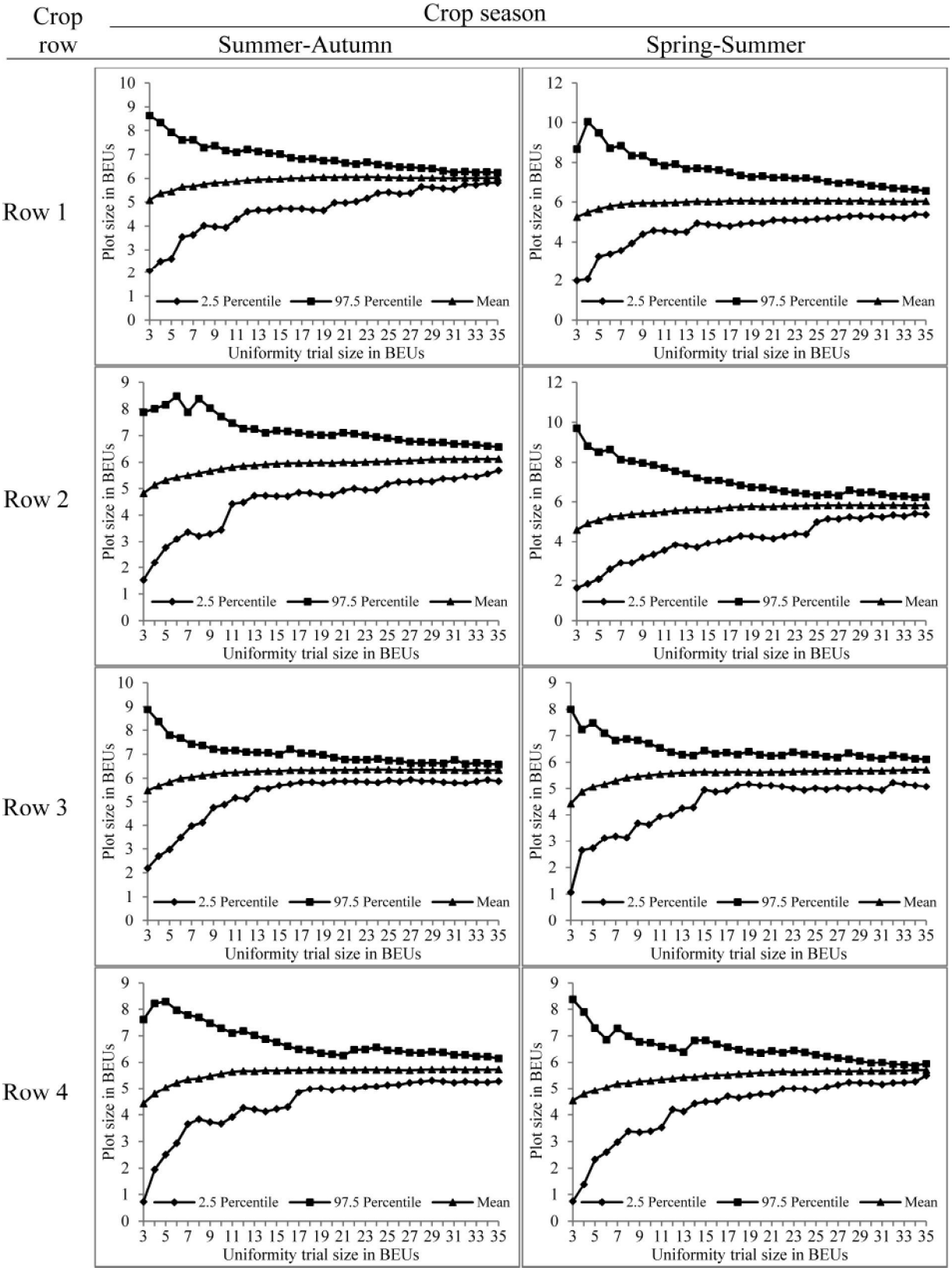
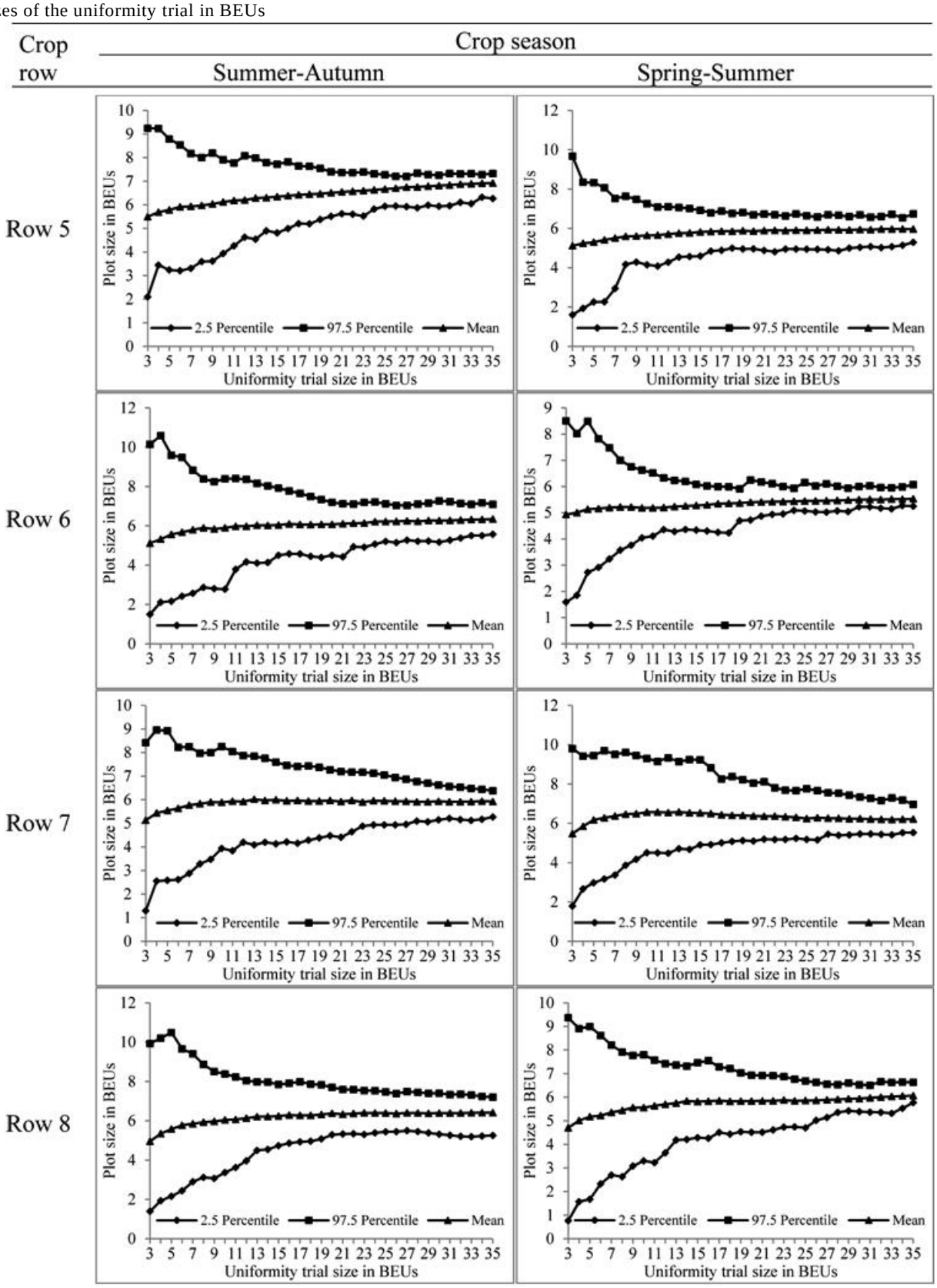


Figure 4 - Graphical representation of 2.5 percentile, mean and 97.5 percentile statistics for the 3,000 estimates of optimum plot size (Xo) for fruit weight of sweet pepper grown in a plastic greenhouse, in basic experimental units (BEUs), for different sizes of the uniformity trial in BEUs



estimate the optimum plot size with an amplitude for CI 95% of less than or equal to two BEUs (Figures 1 and 2). Estimates with this level of precision would seem suitable for planning experiments in restrict environments, and uniformity trial having these dimensions are therefore sufficient to get a good estimate of optimum plot size. In practice, the use of uniformity trial of 60 BEUs in plastic tunnels and of 48 BEUs in a plastic greenhouse to estimate the optimum plot size in lettuce can be found in the literature (LÚCIO *et al*., 2011), confirming the importance and the practical validity of the results obtained in this work.

For sweet pepper, regardless of the crop row, in order to obtain an estimate for the optimum plot size with an amplitude for CI 95% for a Xo of less than or equal to two BEUs, uniformity trial with 26 BEUs are needed in the summer-autumn season and with 29 BEUs in the spring-summer season (Figures 3 and 4). In practice, it can be found in the literature that uniformity trial of 70 BEUs are used to estimate the optimum plot size for sweet pepper in plastic tunnels (LORENTZ *et al*., 2005; LORENTZ; LÚCIO, 2009; LÚCIO *et al*., 2004, 2006).

CONCLUSION

Uniformity trial using 27 basic experimental units to evaluate the fresh phytomass in lettuce plants and 29 basic experimental units to assess fruit weight in the sweet pepper are enough to estimate the optimum plot size for an amplitude of the 95% confidence interval of less than or equal to two basic experimental units.

ACKNOWLEDGEMENT

The authors wish to thank CNPq and FAPERGS for their financial support and the grant of scholarships for productivity in research.

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