Spatial, temporal, and species-specific patterns of heterogeneity in growth chamber experiments

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Despite the sophistication of contemporary growth chambers, growing conditions cannot be uniformly controlled during experiments. Uniformity trials with bean (*Phaseolus vulgaris* cv. Spartan) and maize (*Zea mays* cv. Golden Bantam) in the McGill University Phytotron identified three significant sources of variability. First, not even two identically programmed chambers of the same model and from the same manufacturer provide identical growing environments. Second, programmed environmental conditions are not precisely maintained over time even in a single chamber. Third, the growing environment within a chamber has a consistent pattern of spatial variability with poor growth in the chamber corners and best growth in the center. The importance of these effects varies with species and with the parameters measured, but none can be entirely avoided. Good experimental design with replication of treatments across chambers and blocking within chambers can minimize the negative impact of these sources of uncontrolled experimental variability.

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Malgré l'allure sophistiquée des chambres de croissances actuelles, les conditions de croissance pendant l'expérience ne peuvent pas y être contrôlées uniformément. Les essais d'uniformité conduits dans le phytotron de l'Université McGill, avec le haricot (*Phaseolus vulgaris* cv. Spartan) et le maïs (*Zea mays* cv. Golden Bantam), ont permis d'identifier trois sources importantes de variabilité. Premièrement, deux chambres du même manufacturier et du même modèle ne procurent pas des milieux de croissance identiques. Deuxièmement, les conditions environnementales programmées ne sont pas maintenues de façon exacte pendant toute la durée d'expérience, même dans une seule chambre. Troisièmement, le milieu de croissance présente, à l'intérieur d'une chambre, un patron constant de variabilité spatiale avec une croissance médiocre dans les coins de la chambre et la meilleure croissance au centre. L'importance de ces effets varie avec les espèces et les paramètres mesurés. Cependant, aucun des effets ne peut être entièrement évité. Un bon plan d'expérience avec répétition de traitements à travers les chambres et la subdivision de la chambre en blocs expérimentaux peuvent minimiser l'impact négatif de ces sources de variabilité expérimentale indépendante.

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Introduction

The increasing popularity of growth chambers in the last 20 years has been encouraged by two factors: (*i*) the ability to simulate a wide variety of growth conditions independent of conditions prevailing outdoors, and (*ii*) the widely held perception that growth conditions can be precisely and uniformly controlled in commercially available chambers. The few uniformity trials that have been done, however, cast doubt on the reproducibility of chamber growth conditions. In a uniformity trial with bean, Potvin and Tardif (1988) recognized three potential sources of variability in growth chamber experiments: variation within chambers over time (problems of temporal replication), variation between chambers, and the interaction of these two effects. When comparing the growth of soybeans, Lee and Rawlings (1982) also found a high level of variation among as well as within growth chambers. When lettuce was grown in a series of trials involving growth chambers in several laboratories, more variability was actually found among repetitions within a laboratory than among laboratories (Hammer et al. 1978). Such variability in growth chamber experiments has been recognized for a long time (Langhans 1978), but its implications for experimental design in controlled environments are still too often ignored.

Additional variability can also arise when experiments involve plantings of species mixtures. These effects have not been investigated in previous uniformity trials. One of the few uniformity trials concerned with more than one species (Measures et al. 1973) grew the species in separate growth cabinets. From the perspective of using growth chambers for ecological or population biology studies, this represents an important shortcoming. Experiments in these disciplines often involve comparison of several plant species and (or) populations interplanted in a single growth chamber. Knowledge of the sources of variability among replication and of the level of heterogeneity within growth chambers facilitates the choice of the most appropriate experimental design for any given type of experiment. To determine the sources of variability in mixed-species plantings, we ran a uniformity trial in the new Conviron PGW36 growth chambers at the McGill University Phytotron.

The following effects were tested in this mixed-species trial: the chamber effect, the time effect, and their interaction, as well as the effect of block within chamber. The trial provided two levels of resolution. First, it enables us to assess the precision of blocked replication within a growth chamber. This is useful when an experiment involves application of several treatments (e.g., population, nutrient, watering) in a single growth chamber. Second, the trial provided information on the variability existing between chambers and between times (or over time) for the same chambers. Precision of replication between chambers or times is especially important when the treatment factor is applied to the whole chamber as a unit (e.g., temperature, CO_2 concentration, photoperiod). In addition, since the uniformity trial was conducted with both corn and bean, we could analyze the effects of species and the species by environment interaction. With information on the significance of all these potential sources of variability, it is possible

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to decide the optimal experimental design for a variety of experiments in growth chambers (Potvin and Tardif 1988).

Material and methods

The uniformity trial was performed using two new Conviron PGW36 growth chambers equipped with high light and bypass dehumidification options; the light canopy included end lamps and was left in its uppermost position throughout the experiment. Growth conditions were kept constant throughout the trial, monitored continuously by a microprocessor on each chamber, and measured according to contemporary guidelines for controlled-environment studies (Krizek 1982). The temperature regime was 25:20°C; light:dark temperature transitions were linearly ramped over 1 h. These Conviron chambers are equipped with a magnetic flow control valve that continuously modulates the flow of refrigerant, a substantially more precise temperature control system than the traditional one using solenoids. To further improve temperature stability, the chamber compressors were water-cooled by a chilled water line held between 23 and 28°C rather than air-cooled. Air humidity was set at 70% and monitored by a Vaisala Humicap sensor, the most precise and accurate sensor currently available (Kitano et al. 1984; Kitano and Eguchi 1985). Light level was 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 14-h photoperiod; the lighting transition was in two steps (½ lamps, full lamps) coordinated with the temperature transitions. Both chambers were also equipped for CO_2 control, but it was not activated for this experiment; the air vent was fully open to maximize CO_2 replenishment to the chambers, but some CO_2 drawdown is likely to have occurred as the plants neared harvest size (Tischner 1983). Since commercial CO_2 regulation has only recently become available, this CO_2 protocol best reflects current practice in the majority of growth chamber experiments. Ambient conditions around the chambers were held at 25°C and near 50% relative humidity by the Phytotron ventilation and heating systems.

Two seeds of either bean or maize were sown in 12.7-cm pots filled with a 2:1:1 mixture of potting soil, sand, and vermiculite. The maize seed used was *Zea mays* cv. Golden Bantam and the bean was *Phaseolus vulgaris* cv. Spartan. Shortly after emergence, seedlings were randomly thinned to one per pot. In each chamber, plants were randomly assigned to 12 blocks of 12 plants each. Each block contained six bean and six maize plants, position being assigned at random. Therefore 144 plants were grown in each chamber for a sample size of $n = 72$ per species. The location of blocks within a chamber and of plants within each block is shown in Fig. 1. Plants were hand-watered every Tuesday, Thursday, Saturday, and Sunday with 20 L of water per chamber. On every Tuesday and Saturday 600 mL of nutrient solution (20:20:20) was added to each of the four watering-cans.

The first replication was planted on June 28, 1988, and harvested on July 27, 1988. A second replication was planted August 2, 1988, and harvested August 31, 1988. Both growth chambers were cleaned between the replications and growth conditions were reset. The height of each plant was measured every week following emergence, namely on July 12, 19, and 26 for the first replication and August 16, 23, and 30 for the second. At the end of each replication, aboveground parts were harvested and dried in an oven at 70°C (Fisher, Isotemp model 655F) to a constant dry weight that was recorded.

To help interpret the results of this experiment, a separate detailed analysis of spatial heterogeneity in physical parameters was made in one of the two growth chambers used. The chamber was filled with 144 empty pots laid out as in the preceding experiment and measurements were made of air temperature, windspeed, quantum flux density, and dewpoint temperature at each pot position. Air temperature and windspeed were measured with a Weathermeasure W-141-A hotwire anemometer, quantum flux density with a LiCor LI-185 meter, and dewpoint temperature with a General Eastern Hygro M1 Pacer dewpoint monitor. Four replicate series of measurements were made in random order by block; these data on the patterns of physical heterogeneity within the growth chamber are summarized as means by block of 12 pots (Fig. 1) to facilitate comparison to the growth responses for bean and maize.

Statistical analyses were first conducted separately for each species. Repeated measurements of height were analyzed by analysis of variance with repeated measurements (ANOVAR). The main effects tested were chamber, time, block, and height, the latter being considered as a within-subject effect. In the analysis, the block effect is nested under chamber and hence no interaction involving chamber and block appears. Huynh–Feldt corrected significance levels (Huynh and Feldt 1970) were considered ($e_b = 0.8197$ and $e_m = 0.9373$ for bean and maize).

Final dry weight was analyzed by the following mixed model ANOVA:

$$X_{ijk(i)l} = \mu + C_i + T_j + CT_{ij} + B_{l(i)} + TB_{jl(i)} + \epsilon_{ijk(i)l}$$

Both the chamber (C_i) and the time (T_j) effects were considered to be random so that results from this experiment could be extrapolated to all chambers of the McGill University Phytotron used at any time. On the other hand, the block ($B_{l(i)}$) effect was considered fixed since only those 12 blocks could be fitted in the chambers. Appropriate denominators were chosen according to the expected mean squares (EMS) computed following Cornfield–Tukey (Winer 1971). While the chamber and time effects were tested over the chamber by time EMS, the block effect was tested over the time by block interaction. Both interactions were tested over the error variance. The percentage of the total variance due to each effect was calculated after Winer (1971).

A second series of statistical analyses was conducted on dry weight to assess the effect of environment, genotype (i.e., species), and that of the genotype by environment interaction. In that analysis, the environment was defined as blocks and consequently assessed the responses to the heterogeneity within chambers. We chose to define the environment in this way because the analysis of dry weight, singly for each species, had shown that block was the main, if not sole, source of variation for that parameter. The model fixed under consideration in both analyses was

$$X_{ijk} = \mu + E_i + S_j + ES_{ij} + \epsilon_{ijk}$$

where E_i is the effect of the i_{th} environment (blocks), S_j is the response of species a and ES_{ij} is the interaction effect between environment i and species a.

Following Potvin and Tardif (1988), the significance level was set to $p < 0.25$ for both ANOVARS and ANOVAS to avoid type II error in drawing statistical inferences. The magnitude of experimental effects was estimated following Winer (1971). In the second replication, maize plants failed to germinate in block 9 of one chamber, consequently that block was deleted from all analyses to keep the design balanced.

Results

Beans

The ANOVAR on height of beans reveals that three between-subject effects are statistically significant (Table 1). These effects are the chamber and block main effects as well as the time by block interaction. A look at the within-subject effects further indicates that with the exception of the height by time interaction, all effects are significant at the 0.25 level. These results indicate that height was different between chambers and repetitions and that height also varied among blocks within a chamber. Since the height by chamber by time and the height by block by time interactions were significant, variability related to the repetitions was present, although the time main effect was not significant. The cell means show that overall, bean plants grew taller in chamber 14 than in chamber 9, reaching 25.5 cm compared to 23.0 cm (Table 2).

Examination of height for each of the 12 blocks in chambers 9 and 14 reveals a consistent pattern of spatial heterogeneity.

TABLE 1. Repeated measures analysis of variance for height increment of bean and maize plants

Sources	Bean MS	df	F	p	Maize MS	df	F	p
				Between subject				
C	218.97	1	13.02	<0.25	719.17	1	21.94	<0.25
T	36.53	1	2.17	>0.25	1805	1	55.07	<0.10
B(C)	194.87	22	2.57	<0.05	347.93	20	8.05	<0.0001
C×T	16.82	1	0.49	0.4831	32.79	1	6.18	0.4463
T×B(C)	75.89	22	2.23	0.0018	43.23	20	0.77	0.7507
Error	34.10	239			56.32			
				Within subject				
H	13 237	2	922	<0.0001*	99 359	2	3934	<0.0001*
H×C	143.88	2	10.02	0.0002*	20.22	2	0.80	0.4425*
H×T	16.46	2	1.15	0.3108*	41.34	2	1.64	0.1975*
H×B(C)	76.35	44	5.32	<0.0001*	93.73	40	3.71	<0.0001*
H×C×T	29.91	2	2.08	0.1352*	68.23	2	2.70	0.0719*
H×T×B(C)	31.11	44	2.17	0.0002*	25.46	40	1.01	0.4596*
Error	14.35	478			25.25	438		

NOTE: The ANOVAR model and acronyms for the sources of variation are described in Materials and methods.
*Huynh–Feldt corrected significance levels (see Materials and methods).

TABLE 2. Mean height (cm ± SD) for bean plants grown in 12 blocks in both chamber 9 and chamber 14

Block	H_1	H_2	H_3
Chamber 9			
1	10.86 ± 1.35	14.07 ± 1.92	18.71 ± 4.99
2	10.53 ± 1.21	16.43 ± 3.96	21.43 ± 4.92
3	10.56 ± 0.91	13.44 ± 3.23	17.53 ± 8.26
4	11.27 ± 1.12	15.73 ± 4.41	20.39 ± 5.72
5	10.49 ± 1.18	19.50 ± 4.60	28.77 ± 7.63
6	10.76 ± 0.83	17.28 ± 5.47	23.81 ± 8.92
7	10.34 ± 1.33	16.67 ± 2.59	24.34 ± 3.92
8	10.48 ± 1.01	20.83 ± 4.82	29.01 ± 7.52
9	11.95 ± 2.47	16.55 ± 4.57	20.00 ± 6.09
10	11.06 ± 1.41	16.25 ± 5.00	20.11 ± 5.93
11	10.82 ± 1.79	21.71 ± 7.55	29.87 ± 4.92
12	9.54 ± 1.70	15.36 ± 5.17	17.53 ± 8.26
Chamber 14			
1	10.82 ± 0.73	15.62 ± 2.32	21.47 ± 3.75
2	10.97 ± 1.47	19.10 ± 2.40	30.36 ± 7.65
3	10.90 ± 1.88	14.67 ± 2.05	19.92 ± 4.57
4	10.78 ± 0.91	17.86 ± 5.24	25.13 ± 6.70
5	10.77 ± 2.20	20.18 ± 4.94	30.93 ± 7.71
6	11.25 ± 1.49	16.33 ± 3.15	22.42 ± 8.23
7	10.36 ± 1.08	14.62 ± 2.83	23.55 ± 5.70
8	10.09 ± 1.17	21.45 ± 3.53	36.85 ± 8.92
9	10.93 ± 1.33	17.59 ± 4.56	23.65 ± 5.59
10	9.97 ± 1.29	16.49 ± 3.35	19.43 ± 4.80
11	10.57 ± 1.56	19.65 ± 3.86	31.62 ± 10.18
12	10.18 ± 1.62	16.46 ± 2.76	21.84 ± 4.17

NOTE: Height was measured after 24 (H_1), 36 (H_2), and 48 (H_3) days from planting.

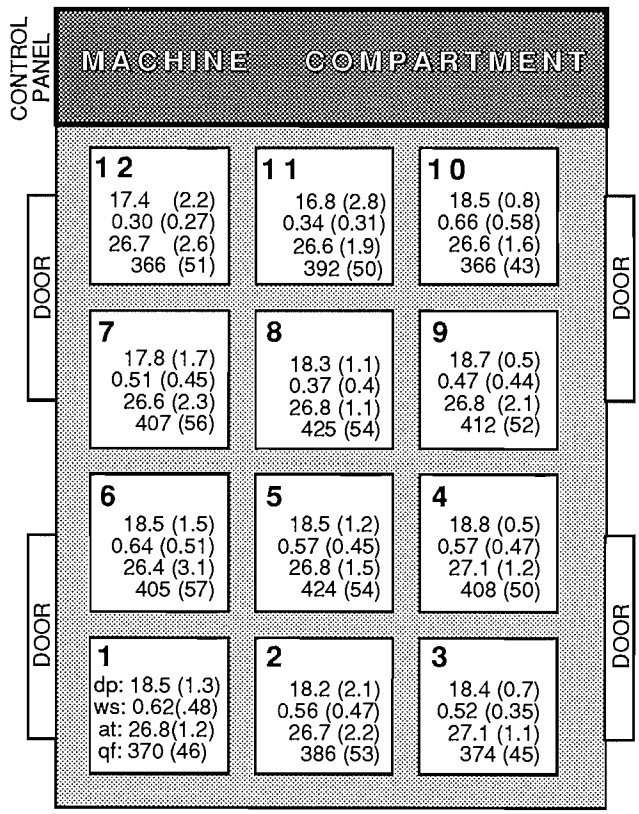

FIG. 1. Positions of blocks within chambers with reference to the Conviron PGW36 control panel and machine compartment. As shown in block 1, each block gives mean values ± SE for dewpoint (*dp*), windspeed (*ws*), air temperature (*at*), and quantum flux (*qf*), as measured in the 12 pot positions, to illustrate the within-chamber variation in physical parameters.

Plants from the four blocks located at the chamber corners (blocks 1, 3, 10, 12; Fig. 1) were always smaller than those of other blocks. The tallest plants were consistently found in blocks 5 and 8 in the center of the growth chamber. The time by block interaction is due to minor variations on this basic pattern of spatial heterogeneity. For example, final height was greatest in block 2 in replication one but in block 8 for the second replication. When the contribution of each main effect to the total variance in height is analyzed (Winer 1971), it appears that the chamber effect was the cause of 3.3% of the variance while the contribution of blocks to the total variation is 9.5%.

Split-plot ANOVA on aboveground dry weight of bean plants indicates that significant sources of variation are due to the time effect ($F = 7.54$, df 1,1, $p < 0.25$) and to the block

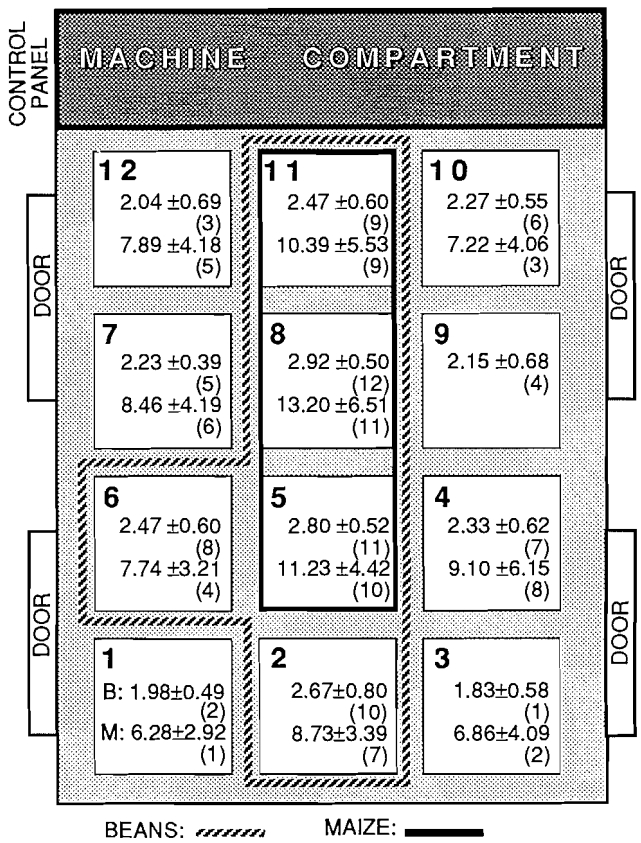

FIG. 2. Aboveground dry weight (g) for plants of beans and maize (B and M in block 1) when grown in 12 blocks within a chamber. Blocks are identified by a number in the upper left corner, while dry weights of each species within the entire block are ranked from smallest (1) to largest (11, maize; 12, bean) by a number in parentheses. Isometric lines for bean and maize separate dry weights that are above and below the distribution mean for each species.

effect ($F = 6.73$, df 20,20, $p < 0.25$). This effect represents 30% of the total experimental variance. In parallel with height results, the dry weights of plants grown in blocks 1, 3, 10, and 12 were always smaller (Fig. 2). Conversely, the heaviest plants were found in blocks 5 and 8 at the center of the chambers.

Maize

The results for maize differ slightly from the results obtained for beans. In maize, as in beans, both the chamber and block between-subject effects were statistically significant (Table 1), but there was a significant main effect of time and no significant block by time effect. On the other hand, height increment in maize, as analyzed by the within-subject effects, was not affected by the chamber nor by the time by block effects. Cell means for maize plants indicate that plants grew tallest in chamber 9 (69.0 cm compared with 68.0 cm) and that they were smaller during the first replication. Spatial patterns of height variation among the blocks in maize (Table 3) are the same as those observed for beans. The plants in the four corners did not grow as well as plants at other locations. Blocks appeared as a major source of variation (29.7%), while variation due to chamber and time represent 4.4 and 11.4% of the total variance, respectively.

Analysis of maize dry weight (Fig. 2) indicates that the only significant sources of variation were the chamber by time interaction ($F = 10.49$, df 1,217, $p < 0.25$) and the block effect ($F = 2.80$, df 20,20, $p < 0.25$). Overall cell means suggest that the significant chamber by time interaction is due to the large dry weight achieved by maize plants when grown for the second time in chamber 14. This interaction is responsible for 3% of the experimental variance while the block effect explains 1.2% of the total variance. Patterns of spatial variation within chambers are once again similar to all previous results. Plants achieved a smaller aboveground dry weight when grown in the four corner blocks and final weight was largest for blocks 5 and 8 (Fig. 2).

TABLE 3. Mean height (cm ± SD) for maize plants grown in 11 blocks in both chamber 9 and chamber 14

Block	H_1	H_2	H_3
Chamber 9			
1	31.13 ± 5.22	53.23 ± 6.76	62.80 ± 10.59
2	31.49 ± 3.39	54.23 ± 4.77	70.49 ± 7.02
3	32.05 ± 3.55	52.79 ± 3.94	65.34 ± 6.26
4	32.41 ± 5.22	56.82 ± 5.04	69.42 ± 6.29
5	33.28 ± 6.81	59.94 ± 4.34	80.35 ± 9.13
6	32.46 ± 6.03	55.57 ± 7.31	70.24 ± 9.36
7	30.01 ± 6.03	54.53 ± 5.86	68.78 ± 9.31
8	30.46 ± 2.89	57.58 ± 4.05	77.57 ± 9.36
10	29.13 ± 6.37	49.80 ± 9.18	58.09 ± 8.77
11	30.17 ± 4.15	57.75 ± 5.44	72.21 ± 8.25
12	29.01 ± 2.79	53.79 ± 4.12	67.08 ± 5.24
Chamber 14			
1	27.34 ± 2.92	48.39 ± 5.71	60.89 ± 6.18
2	30.08 ± 3.29	54.53 ± 3.80	68.51 ± 4.59
3	26.10 ± 6.45	48.23 ± 6.43	60.08 ± 7.95
4	27.50 ± 6.03	53.93 ± 3.33	69.86 ± 12.95
5	31.18 ± 5.38	53.91 ± 4.98	74.87 ± 3.84
6	30.98 ± 5.05	54.00 ± 4.91	67.64 ± 7.03
7	28.87 ± 4.09	53.51 ± 4.46	73.10 ± 9.11
8	30.30 ± 3.35	53.37 ± 4.68	75.81 ± 5.26
10	28.23 ± 5.30	51.47 ± 6.33	64.43 ± 7.37
11	30.53 ± 6.24	55.79 ± 6.09	70.71 ± 4.91
12	28.99 ± 3.28	51.88 ± 8.04	61.20 ± 6.43

NOTE: Incomplete germination forced the elimination of block 9 from the analyses.

Physical heterogeneity in the chamber environment

The most notable aspect of the within-chamber environmental pattern is the lower quantum flux densities near the walls compared to the center of the chamber. This is most marked at the two end walls despite the extra lamps designed to minimize this effect. Other parameters are reasonably uniform with the exception of lower dewpoints and windspeeds in the control panel corner of the chamber (Fig. 1). These differences are probably attributable to the difficulties of exactly equalizing air flows within the chamber. Air flows into the chamber from a plenum beneath the perforated floor with the airflow driven by two squirrel cage fans in the machine compartment. The two fans must be balanced and a system of baffles below the floor correctly positioned to equalize flow to all parts of the chamber; the exact balancing of flow when the chamber is full of pots is unlikely to occur without special and time-consuming adjustments. This type of heterogeneity in wind and humidity, while of less predictable pattern than that for quantum flux, is likely to occur in most chambers under normal operating conditions.

Species and environment effects

Analysis of variance for the effects of species, environment, and their interaction (Table 4) indicates that at either the

TABLE 4. Analysis of variance for dry weight of bean and maize when the environment is defined as blocks within chambers

Sources	MS	df	F	p
Species	5513.24	1	520.60	<0.0001
Environment	66.90	10	6.32	<0.0001
S×E	36.77	10	3.47	=0.0002
Error	5295.04	500		

NOTE: This analysis complements the ANOVAR (Table 1) by testing species differences in response to the environmental heterogeneity within chambers.

between-replication or the within-chamber levels, all effects are statistically significant. As would be expected the strong species effect was due to the larger dry weight of maize plants; dry weight in the 12 blocks ranged between 1.8 and 2.9 g for bean and 6.3 and 13.2 g for maize. The mean dry weights are 2.4 g for bean and 9.7 g for maize. The species by environment interaction is explained by slight differences in the pattern of variation among blocks (Fig. 2). For example, the smallest overall dry weight recorded for maize is found in block 1 while it is found in block 3 for bean. For both species, however, the largest aboveground dry weight was obtained from block 8.

Discussion

Our results indicate that experiments using even the most sophisticated of contemporary chambers can still not be assumed to be free of uncontrolled variations in growing conditions. Because such uncontrolled variations usually will affect the response of plants, any experiments analyzing treatment factors applied to a growth chamber as an experimental unit should be replicated by using at least two chambers per treatment or by repeating the experiment. Such replication is the only way to avoid confounding of treatment and chamber effects. Since the growth of maize and beans was affected by both the chamber and a time-related effect, replication ideally should be conducted both in space and in time. It is worth noting that the importance of this between-chamber or replicate effect depends on the parameters being measured; dry weight is less responsive to the sources of variation in growth chambers than is height increment. Potvin and Tardif (1988) also found similar parameter-dependent responses in their single-species uniformity trials with *Phaseolus vulgaris*. Therefore, anyone monitoring a number of parameters should assess the sources of variability for each of them and allow for this variability in designing and interpreting experiments.

Since ideal levels of replication will not always be feasible, it is useful to consider the possible causes of the chamber and time effects so that variability can be minimized as much as possible. The chamber effect can be largely attributed to intrinsic characteristics of the Conviron chambers themselves, characteristics that are typical of contemporary designs for all commercial growth chambers. The design and construction of chambers from even a single manufacture change over time as models are improved or altered because of the availability of materials. Even if two chambers are identical in construction, their location in relation to room ventilators, sources of chilled water, and similar services can influence their performance. The variation due to such differences among chambers can be accounted for most easily by replicating an experiment simultaneously in at least two chambers. The variations in a single chamber over time arise from diverse sources that are difficult to control (changes in personnel, seasonal shifts in the ambient conditions of the room housing the growth chambers, spectral changes associated with normal aging of lamps, power outages, pest outbreaks, and the like). The best way to account for these diverse sources of temporal variation is to replicate an experiment in time. Careful attention to experimental protocols and regular maintenance of equipment can help reduce temporal variation when replication is impractical.

The single greatest source of variability found in this uniformity trial was actually the within-chamber block effect, an effect of chamber design that cannot readily be removed. For the two species considered, the contribution to the total variance in overall height due to the block effect is the largest of all effects, 10 and 30% for bean and maize, respectively. Fortunately, these patterns of spatial variation are rather constant between chamber and time as well as for species and parameters measured and thus can be allowed for in designing experiments. In the Conviron PGW36 chambers, plants always did poorly when grown in a corner while the best growth was achieved in the two central blocks. This is probably due to lighting and air circulation patterns inherent in the design of the chambers themselves (Fig. 1). The presence of such a consistent and strong spatial effect within chambers indicates some elementary rules that must be followed when analyzing certain within-chamber treatment factors (e.g., nutrient, drought stress). Any level of such treatments should be applied at least twice within each chamber to enable separation of the position and of the treatment effect in the statistical analysis. If the experimental variance due to block can be analyzed, it will reduce the error term and consequently enhance changes to discern treatment effects. On the other hand, the poor growth of plants in the four corners of the chambers suggests that it might be best to leave that space unused as much as possible; plants should be grown there during an experiment to assure stability of the spatial pattern, but these corner plants should ideally not be used for data-taking.

The results of the present experiment also provide an additional and novel dimension to uniformity trials by comparing two species grown together in the same environments. The analysis indicates the presence of strong species by environment interaction. Consequently, it is unrealistic to assume that the response of different species and (or) populations within a growth chamber environment will be constant. However, the difference between the two species stems from small variations of a more general pattern. Both species did poorly in the corners and better in the center, although the relative ranking of the corners varied between species (Fig. 2). Therefore, the effects of within-chamber heterogeneity can be controlled by blocking, provided that both species are present in each block. The possibility that similar effects may occur in comparing populations or genotypes of a single species should also be considered in the design of experiments in controlled environment cabinets.

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