Plant and Soil VI, no 4 August 1955

TECHNIQUES FOR EXPERIMENTS WITH POT PLANTS

by W. J. C. LAWRENCE

John Innes Horticultural Institution

INTRODUCTION

From time to time, attention has been directed to the special problems of experimentation with pot plants in glasshouses (Le Clerg[1]), Youden[2]), Cox and Cochran[3])). It has been pointed out (Federer[4])) that (1) the magnitude of the variations encountered in greenhouse experiments often exceeds that observed in field experiments, and (2) all too frequently experimenters assume that the variation in the greenhouse is small enough to be neglected. Very few people have attempted to measure the magnitude and distribution of climatic variables in glasshouses but those who have are well aware of the truth of Federer's remarks. The problem of satisfactory experimental design is further complicated by the lack of space, especially useful space, in a glasshouse, compared with out-of-doors.

In the course of the author's investigations into glasshouse design, seed and potting composts, and methods of propagation (Lawrence[5])), it became apparent that standardization of cultural and experimental techniques was essential to satisfactory experimentation bearing on the germination of seeds in pots, pans and seed boxes; the growing-on of seedlings in small to medium sized pots, in soil blocks and in seed boxes; and the growing to maturity of plants in medium to large sized pots — all on glasshouse benches.

THE PROBLEM

For experiments in glasshouses there are three major sources of error affecting the behaviour of the plant, (i) its genetics, (ii) the climate, and (iii) the experimental methods. These errors obscure, in varying degree, those very features of plant behaviour the experimenter proposes to study. It is true that correct experimental design will enable an estimate to be made of uncontrollable variations 'in order to decide whether the treatment differences can reasonably be attributed to random fluctuation or not' (Fisher[6]). But as Fisher also points out, refinements in experimental technique 'may be important and even essential, in permitting the phenomenon under test to manifest itself'. The object, therefore, of this paper is to deal with the refinements of experimental techniques as an aid to critical, convenient and economic experimentation. Examples will be given of some of the sources of error in glasshouse experiments. The results of applying the techniques described in the present account are given in the paper that follows (Alvey[10]).

GENETICS

Errors arising from differences in plant behaviour due to genetical factors, can be reduced by using inbred or homogeneous material, *e.g.* hybrid maize, tomato, (Lewis[7]). With high genetical uniformity, differences in growth due to treatments are generally more readily seen and measured. If it is necessary to use species which are outbreeders, or strains that are heterogeneous, *e.g.* brassicas, then some uniformity may be obtained by selecting equal-sized seedlings when pricking-out. This may prevent treatment differences from being obscured by gross genetical variance.

CLIMATE

Uncontrolled variations in light, temperature, humidity and air displacement are frequent sources of error in glasshouse experiments.

1. *Light*

Persistent differences in light intensity of up to a hundred foot-candles or more, commonly occur in glasshouses unperceived by, and therefore unknown to, the research worker. Since within limits as wide as 150–1,500 foot-candles growth of most plants is proportional to the intensity of light during unit time, it will be realised that differences in light intensity within the glasshouse may and do contribute to errors in glasshouse experiments.

The chief causes of light gradients are 'shadows' cast by (*1*) nearby glasshouses, buildings, trees, *etc.*, (*2*) the structure of the glasshouse itself, (*3*) tall plants near the experiment. Shadow from (*1*) can be avoided by good planning and from (*3*) by simply moving the tall plants further away.

Shadow from (*2*) requires consideration. In a moderately long glasshouse orientated in a north-south direction, heavy shadows are cast by the roof structure during the middle hours of the day,

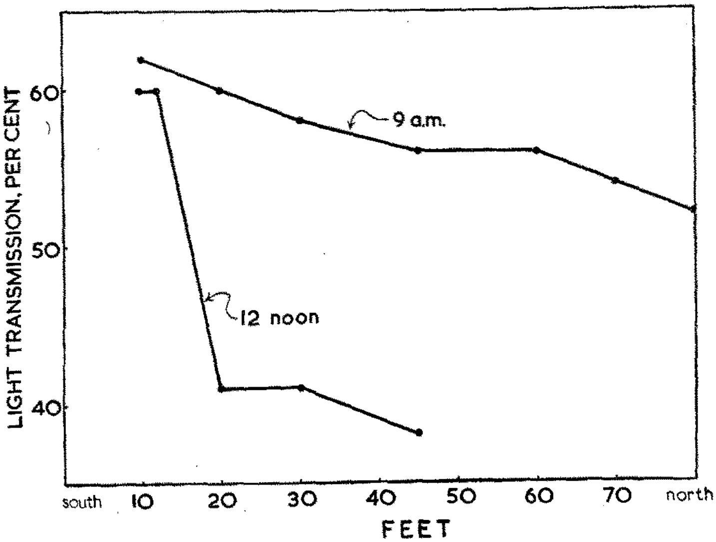

Fig. 1. Showing the light intensity gradients at bench level along the middle of a glasshouse 86 ft. × 20 ft., orientated in a north-south direction. The intensity of light in the glasshouse is expressed as a percentage of the light outside. Measurements made with a Weston selenium cell photometer on 31st December, at Merton.

the shadow being least at the extreme south end. Thus, during a large part of the hours of daylight, a steep light gradient runs northward from the south end of the house, its length depending on the season, *i.e.* the height of the sun (Figs. 1, 2). A similar but less marked gradient often exists even when the sun is not shining, though it is then rarely perceived. Gradients may also occur elsewhere than the south end of a north-south house, *e.g.* across the house (Fig. 2). The effect of such light gradients on the weight and habit of lettuce can be seen from Fig. 3.

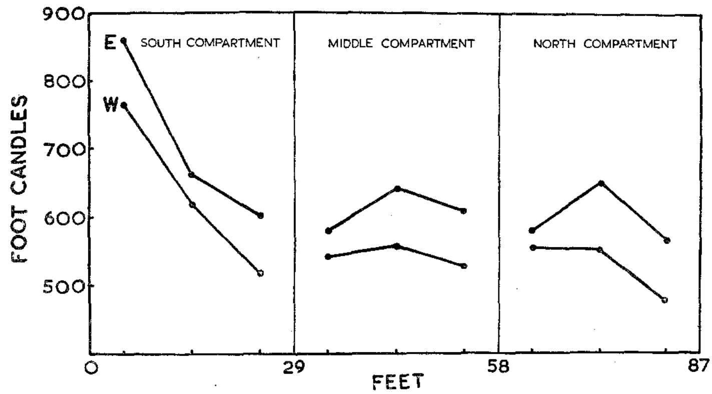

Fig. 2. Showing the light intensity gradients at ground level in the east member of a pair of 'ridge and furrow' glasshouses, orientated in a north-south direction. The house is divided transversely into three compartments by glass partitions. A path runs down the centre of the house. E = the eastern border, W = the western border, adjacent to the second house. Measurements made at 2 hourly intervals, 8 a.m. – 4 p.m., from 8th to 28th October, at Merton.

Most of the difficulties associated with a north-south orientation disappear when the orientation is east-west (Fig. 4). In winter time,

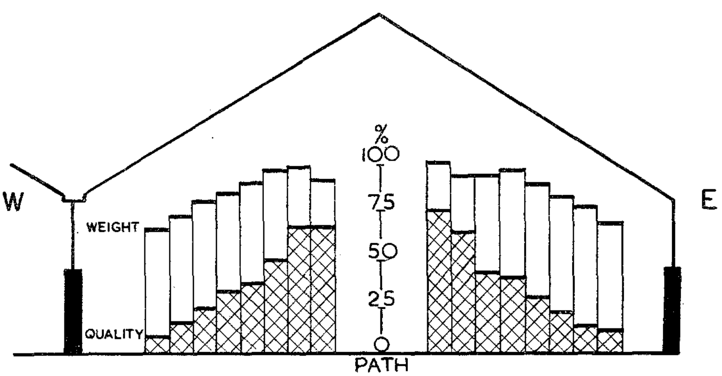

Fig. 3. Showing weights and quality of lettuce 5B grown as a winter crop in the glasshouse referred to in Fig. 2.

in particular, the greatest uniformity in light intensity is found in the single east-west house with high eaves, glazed with large glass supported on a slender structure with no obstructions to the south of the house and to the north no other glasshouse nearer

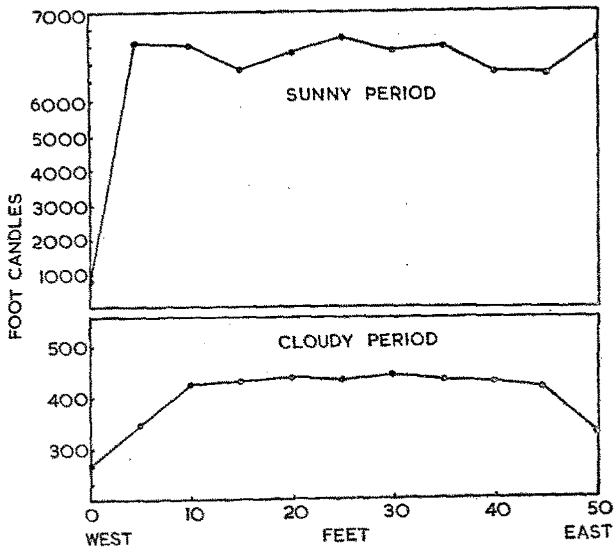

Fig. 4. Showing uniform distribution of light intensity in a glasshouse with east-west orientation. Measurements made on two occasions, both at 3 p.m., during the last week in August. To save space, the foot-candle intervals (ordinate) have been contracted at the lower intensities.

than 20 ft. or so. Such a house is comparatively free of light gradients except at the extreme ends and these can easily be avoided in the lay-out of experiments.

2. *Temperature*

Horizontal gradients in the ambient temperature of glasshouses almost always occur; and sharp gradients have been found to occur on benches within so little as 3 to 5 feet (C o x and C o c h r a n [3])). One such gradient was observed in the course of temperature measurements in a propagating house at Bayfordbury. Two thermometers were suitably exposed 4 in. above the middle of a bench $3\frac{1}{2}$ ft. wide and 48 ft. long, at distances of 19 and 29 ft. from one end of the bench. The mean minimum temperatures over two successive periods of three weeks in October–November were found to be 58.0°F and 56.1°F, and 56.7°F and 54.4°F respectively. Thus a persistent difference of 2°F was shown to exist between two points only 10 ft. apart, a difference large enough to affect seedling growth appreciably. The cause of this gradient was traced to excess of heat from sub-mains entering one end of the house.

A gradient along the bench is generally the consequence of failure to balance heat losses from the ends of the house by the correct length (surface) of heating pipe. Thus at Merton, where one gable end of a house was fully exposed to wind and the other end protected by an adjoining glasshouse, a mean difference of 5°F in the minimum temperature was found to occur in a distance of 40 ft. Such differences can be corrected by running extra pipes across the exposed gable end and, if necessary, insulating a few feet of pipe at the protected end. In gravity hot water systems the pipe temperature may be 40–50°F lower at the distant end of a glasshouse owing to the sluggish flow of the hot water. The installation of a water circulator is an easy method of rectifying this defect.

Temperature gradients across a glasshouse bench arise from (*1*) proximity to the side of the house, especially where there are ventilators, and (*2*) warm air currents rising from heating pipes beneath the bench and flowing around its edges. In the first case, tight fitting ventilators will reduce the temperature gradient; and the provision of ventilators that come no lower than half way down the side of the house will reduce the gradient still further. In the second case, a clear space of at least 6 in. should be left between the back of the bench and the side of the house to permit warm air to rise from the pipes on the side wall and thus offset the temperature drop near the glass. Even then, for example, in a house controlled at 60°F the mean temperatures at 7 in. and 1 in. from the glass may be of the order 57°F and 53F° in winter time. Careful positioning of the heating pipes beneath the bench will help to reduce edge effects.

The temperature gradient across a 6 ft. wide middle bench is likely to be smaller than that across a 3 ft. wide side bench. Vertical gradients are, as a rule, of little consequence to bench plants. Broken, slipped or cracked panes of glass anywhere in the house are potent causes of local differences in air temperature. Repairs should be immediate and thorough. Equally, ill-fitting ventilators should receive prompt attention.

Once temperature gradients have been reduced to a minimum, maintenance of a uniform air temperature (so far as artificial heat is concerned) can be achieved by means of a thermostat. An efficient heating system should respond quickly to changes in glasshouse temperature induced by solar radiation, wind, *etc.* Electricity, steam, rapidly circulated hot water in 1–2 in. pipes, all give a rapid response to changes in air temperature. A satisfactory thermostatically controlled heating system will, at any given moment, give the required temperature within $\pm$ 2°F and, over periods of hours or months, a very close agreement to the set mean. For example, during one winter, with the night thermostat set at 60°F, the mean night temperature was 59.9°F ($\sigma = 0.70$°F). Similarly, in another glasshouse, the mean was 59.5°F ($\sigma = 0.66$°F).

It should be especially noted that good temperature control necessitates good installation of thermostats and thermometers. Errors of 5°F–10°F have been found to be common on sunny days unless the instruments are adequately screened from direct solar radiation. The larger instruments, *e.g.* thermographs, are particularly unreliable. It is also necessary to ensure that thermometers are adequately aspirated, *i.e.* they must be spaced an inch or so from both screening and supporting materials so that air can move freely past the bulb or sensitive part.

3. *Humidity*

Humidity is difficult to control in glasshouses. Fortunately, where temperature gradients have been minimised the only humidity gradients likely to upset experiments are those created by plants themselves when grown in number on a bench or in the ground. Differences in seedling growth which might arise from these local humidity conditions can be overcome by (*1*) avoiding overcrowding of the plants, (*2*) selective "damping-down" of those parts of the bench which frequently dry out *).

4. *Ventilation*

Ventilation on the lee side of the house and a careful watch for sudden changes in the direction of the wind will help to reduce draughts. But on hot, windy days it is impossible to cool a glass-

*) A 1-in. layer of $\frac{3}{8}$-in. graded and weathered clinker on the bench will greatly help to secure and maintain useful humidity conditions.

house adequately by ventilation alone. Tiffany (cotton), cane, lath or plastic blinds, external to the house, are essential for reducing the effects of sunshine and for making ventilation more manageable. A blind that reduces light to 30 per cent of the outside intensity has been found to be satisfactory.

For cooling purposes, fan ventilation or semi-air-conditioning are inadequate (and costly) for any but quite small glasshouses (or compartments) and even then blinds may be necessary in bright, sunny weather.

EXPERIMENTAL METHODS

These relate to the handling of materials and plants in the preparation of experiments, to the day-to-day care and cultivation of the plants and to the measurements on which the results are based.

1. *General*

i. In preparing experiments certain operations are common to *all* treatments. A given operation should be performed by one man only throughout all treatments, *e.g.* the measuring of bulk compost ingredients, pricking-out, potting.

ii. Ideally, one man should handle the whole of the operations. If, in large experiments, two or more men must be employed, then the principle stated above can be extended, thus. Any operation specific to *one* treatment should be shared equally by the two men.

iii. Standardized methods in definite sequence should be employed consistently so that they become a habit with staff, *e.g.* instruction sheets, prepared labels, measuring of materials.

2. *Composts*

Large variations in plant growth and development may result from the improper making and handling of seed and pottings composts, not only within an experiment, but between successive experiments. Most of this variation can be avoided by using the standardized John Innes Composts.The full details for making these are described elsewhere (Lawrence and Newell[8])). For experimental work the following points are of special importance:—

i. Mixing. This should be very thorough. Fertilizers should first be mixed with some or all of the sand (which must be dry) and then the fertilized sand thoroughly incorporated with the bulk compost ingredients.

ii. Treatments. When compost is required in two or more lots constituting the treatments to be investigated, uniformity of bulk materials can be ensured by division and sub-division of one original heap of compost.

3. *Pots and boxes*

Clay pots differ in shape, capacity and porosity from one maker to another. These differences may introduce errors in plant growth, therefore pots should be uniform in a given experiment. Thus, in a comparison between two makes of pot of the same size, the weight of one month old tomato plants was 8 per cent greater ($P > 0.001$) with one make of pot than the other. Pots which have been used previously should be thoroughly washed, inside and out, and then immersed in clean water for two days or so. This is a precautionary measure aimed at reducing gross differences in the soluble nutrient contents of the pots. Significant differences in plant weight can arise from the nutrient content of used pots. Glazed pots may be employed, of course, but they are much more costly. Although the author has not investigated variation introduced by previously used boxes, thorough brushing-out is obviously desirable. For the most critical work new boxes should be employed.

4. *Soil blocks*

The chief cause of errors when soil blocks are used is variable soil compression. Uniformity can be secured by seeing that the same amount of soil is fed into the mould for every block. Some machines always make uniform blocks; others require the operator to make adjustments or contrive an even flow of soil to the mould.

5. *Amount of soil*

Weighing or special measuring of the soil can be avoided by using vessels of equal capacity, pouring the soil loosely into the pot or box until it is slightly overfull, then striking-off the surplus soil with a flat piece of wood. The soil may then be compacted if desired. Alternatively, seedlings may be pricked-out straight into the loose soil and watered-in, a method which gives uniform compaction from pot to pot. This is an excellent method with tomato seedlings.

Where crocks must be used for drainage, the most convenient type is the round, slightly convex, perforated metal variety. Potsherds have the disadvantage of requiring sterilization, breaking-up and careful placement. Inevitably, they bulk differently from pot to pot and so alter the volume of soil and, in consequence, the nutrition of the plant. All these disadvantages are avoided by the use of metal "crocks".

6. *Compaction and moisture*

Undue compaction of soil that is too moist, may lead to marked retardation of seedling growth and therefore, to the introduction of avoidable errors. This danger is all the greater if the inherent physical condition of the compost is mediocre. Soil for use in pots and boxes should be in "potting condition", *i.e.* it should just bind or mould when gripped hard in the hand but readily fall apart when the grip is loosened and the soil slightly jolted. If the soil is in potting condition most of the error from differences or compaction will be avoided. With a properly made compost there is no advantage in making the soil very firm. Moderate or slight compaction is enough and this can be obtained by the judicious use of the fingers or by shaped "pressers" which fit the pot or box.

7. *Labelling*

Errors from wrong labelling are obviously among the most serious which can arise since they involve confusion of treatments. If possible, labels should be inserted into pans, pots, *etc.*, before seed is sown or seedlings pricked-out. Preferably, only the one pan or box supplying seedlings for a single treatment should be on the potting bench at a given time and this pan or box should be removed to a distance before the next treatment is dealt with.

8. *Seed sowing*

The aim should be to ensure uniform and rapid germination of the seeds. Incorrect soil texture, compaction, depth of sowing and covering of the seeds are all potential causes of variation in germination and seedling growth. Good texture can be assured by using John Innes Compost which has been passed through a $\frac{1}{4}$-in. sieve. For covering the smaller seeds an $\frac{1}{8}$-in. sieve should be used; finer soil encourages surface panning and poor aeration. Compaction becomes a danger when the compost is too moist. It is better to have the compost *slightly* too dry than too moist, since it is then difficult to make it too firm, even with intent. Depth of sowing and covering are matters of experience which any competent gardener should be able to deal with. It is better to sow *slightly* too deep than too shallow.

9. *Watering-in*

In the case of small seeds, thoughtless watering-in often leads to variable germination and growth by washing the seeds about and disturbing the surface of the soil. Seed boxes for sowing should be prepared an hour or two before required, well watered with a rose can and then allowed to drain for 1 to 2 hours in a warm place. After the seeds have been sown and lightly covered with sifted soil, just enough water should be applied with a fine rose can to moisten the sifted soil. The box should then be covered with glass, brown paper, *etc.*, to retard drying-out of the soil.

10. *Pricking-out*

Large errors may arise from differences in the way seedlings are pricked-out (or potted-off, or potted-on) by different gardeners (Warne [9]). For example, in one experiment with lettuce, the yield from plants planted-out by an experienced man was 13 per cent greater than from those planted by an apprentice (Lawrence [5]). Consequently, it is desirable that in a given experiment the pricking-out, *etc.*, should be done by one and the same individual. If the experiment is too large to permit this, then the seedlings constituting a randomised block should be pricked-out by one man.

It is of outstanding importance, amounting to a principle, that, as far as possible, one and the same person handles and cares for the plants in pot and box experiments. Further, if this person is aware of the part he can play in assisting to establish and maintain a high standard of experimentation, he is worth a very great deal to the research worker. Enlightened co-operation of this kind is a *sine qua non* for intensive experimentation. The research worker cannot always be present to supervise the preparation of materials or the growing of plants and it is surprising how often errors may arise, without his knowledge, through failure of gardeners and assistants to appreciate the requirements of critical experimentation.

In pricking-out, errors arise from (*1*) the way the seedling is removed from the seed box, (*2*) the care with which it is put into the new soil, and (*3*) the degree to which the soil is compacted by the pricking-out dibber. Errors from these causes can be minimised by (*1*) pricking-out the seedling as soon as cotyledon expansion has occurred, *i.e.* while the radical is still no more than $\frac{1}{2}$ to $\frac{3}{4}$ in. long and therefore, easily removed with the minimum damage, (*2*) holding the seedling by a cotyledon (never the hypocotyl) and ensuring the hole made by the dibber is large and deep enough, and (*3*) using loose-filled pots in which no compaction of the soil follows pricking-out but watering-in is relied on to produce adequate settling of the soil around the seedling root. This latter technique, wherever it can be practised, also has the merit of standardizing the handling of the soil.

11. *Selection of seedlings*

It is sometimes an advantage to select at the pricking-out stage seedlings all of the same size, *e.g.* when the experiment is to test the effect of soil, method of handling, feeding, *etc.* Obvious weaklings and rogues should, of course, be rejected. In other cases, selection cannot be permitted, *e.g.* in genetical studies. In these cases, if all the seedlings are not required, the best method of avoiding conscious and unconscious selection is to take *all* the seedlings from a narrow band or bands, *e.g.* $\frac{1}{4}$ in. wide, across the seed vessel.

12. *Potting-off and potting-on*

These operations are best avoided, since they materially disturb and retard plant development and introduce errors.

13. *The bench*

A "solid" bench or stage covered with $\frac{3}{8}$ in. graded clinker to a depth of at least one inch is preferable to a slatted bench that allows air currents to pass around the plants, thereby introducing variations in growth. The use of clinker, shingle or similar material on a solid bench is not to be recommended when there is a possibility of roots growing out from the bottoms of pots and boxes. Should this happen, large variations in growth may occur. These can be avoided by growing the plants on the bare bench if this is constructed of rough one inch boards, asbestos-cement sheets, slate, *etc.* Bare boards treated with a preservative harmless to plants are to be preferred to bare slate and asbestos sheet since, in summer time, these mineral materials may become excessively heated by solar radiation to the detriment of plant growth. Further, water evaporates too quickly from a smooth surface such as slate. Rough boards, by comparison, remain moist for a longer time and provide a humid atmosphere around the plants.

At all stages of growth the spacing of pots should be sufficient to prevent the "drawing" which results from overcrowding and the soft growth which is probably an expression of excessive humidity amongst the plants. Spacing in and between the rows should be uniform, though these two spacings need not always be alike. For example, while the requirement for uniform growth might be met by staging young plants pot-thick, the requirements of good plant management (*i.e.* damping-down) might make it desirable to adopt an arrangement whereby the plants were pot-thick in the rows but spaced between them.

It may be noted here that the maximum width for a single bench which permits convenient handling of pot plants is $3\frac{1}{2}$ ft. when the height is not more than $2\frac{1}{2}$ ft. This width allows ten $3\frac{1}{2}$-in. pots to be staged across the bench "pot-thick", or five 5-in. pots, or 4 standard seed boxes. The advantages of the round number ten, are obvious. Alternatively, a row may comprise two lots of five plants. Each row or half row can comprise a plot and n rows a block. It is found that randomised block experiments can be carried out satisfactorily on glasshouse benches.

14. *Watering*

Plants should be watered as uniformly as possible, taking care to avoid both drying-out and over-watering, *i.e.* the ordinary technique of good watering is adequate if applied a little more critically. When plants are to be weighed fresh on a given morning, they should be looked over for watering the previous afternoon or evening so as to have the soil in all pots at "field capacity" and the plants turgid.

15. *Weighing*

In many experiments, especially with young plants, the speediest method of assessing treatment differences is by weighing the fresh aerial portions of the plant (fresh weight) at a given stage in development. Fresh weight is a reliable criterion of growth when the standardized techniques discussed in this paper are employed. A precaution to be observed concerns the stage at which pot and box plants should be cut and weighed. Partial exhaustion of the soil in respect of one or more nutrients, especially nitrogen, can occur without any obvious signs. Thus, in the case of nitrogen a plant may be a fairly good green colour yet suffering from "invisible malnutrition". This malnutrition will be an intrusive variable in certain experiments, therefore, an effort should be made to terminate the experiment before the onset of malnutrition. If feeding is adopted to counter malnutrition it is desirable to use measured doses of a completely water-soluble fertilizer, freshly made up.

The most suitable level for cutting off seedlings depends on the species. Tomato is best cut off immediately below the cotyledons, lettuce at soil level. For the rapid weighing of single young plants, the author uses a series of fine helical springs suspended in large glass tubes to screen off draughts. For the weighing of groups of plants a useful instrument is a semi-self-indicating balance with an adjustable zero.

DISCUSSION

The techniques described in this paper are essentially simple, calling for no great expense, special apparatus, or highly skilled manipulation. They can be used with profit in many experiments concerned with genetical, morphological and physiological studies of plant behaviour. Thus, in the absence of gross environmental effects, the geneticist can more easily see the direct effect of gene action and interaction and measure genetical differences with greater accuracy. Similarly, the physiologist can compare treatment effects with greater ease and certainty and with economy of time and material.

The development of these techniques places a new experimental method in the hands of the research worker, namely, the statistical assessment of quantitative and qualitative variations in plant growth and development.

CONCLUSION

Good growing facilities are inseparable from good experimentation. This principle is not always recognised in experiments involving the raising of plants in pots and boxes under glass. Commonly the general climatic conditions of the glasshouse are far from ideal with very little control attempted or achieved. And the preparation and carrying through of such experiments is sometimes almost casual so far as uniformity and consistency of technique are concerned. A criterion and standard which has much to commend it may be stated as follows. Good experimentation should satisfy equally the skilled and knowledgeable gardener and the critical and experienced research worker.

SUMMARY

Simple, standardized techniques for the raising of pot plants on glasshouse benches are described. The techniques are especially suitable for the statistical assessment of quantitative and qualitative variations in plant populations by the geneticiest, plant breeder and plant physiologist.

Received September 27, 1954.

REFERENCES

1) Le Clerg, E. L., Factors affecting experimental error in greenhouse pot tests with sugar beet. Phytopathology **25**, 1019 (1935).
2) Youden, W. J., Experimental designs to increase accuracy of greenhouse studies. Contrib. Boyce Thompson Inst. **11**, (1939–41).
3) Cox, G. M. and Cochran, J. W. G., Designs of greenhouse experiments for statistical analysis. Soil Sci. **62**, 87–98 (1946).
4) Federer, W. T., Plot technique in greenhouse experiments. Proc. Amer. Soc. Hort. Sci. **62**, 31–34 (1953).
5) Lawrence, W. J. C., Science and the glasshouse. Oliver & Boyd Ltd., Edinburgh. 15s (1950).
6) Fisher, R. A., The design of experiments. Oliver & Boyd Ltd., Edinburgh. 13s. 6d. (1935).
7) Lewis, D., Gene-environment interactions. Heredity **8**, 333–356 (1954).
8) Lawrence, W. J. C. and Newell, J., Seed and potting Composts. George Allen & Unwin Ltd., London. 8s. 6d. (1952).
9) Warne, L. G. G., The personal factor in thinning. The Fruit Grower, No. 2, 819, 28 (5 January, 1950).
10) Alvey, N. G., Error in glasshouse experiments. Plant and Soil **6**, 347–359 (1955).