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Experiment: Methodological Basis

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Abstract: ***Reference books usually present experiment designs as recipes, one of which should be chosen for each particular experiment. This approach extends to teaching and leads the researcher to understand that he is limited to the use of these experiment designs. The consequences are the underestimation of the planning of the experiment, the adaptation of research plans to these designs and the formulation of experiment structures inadequate to achieve the research objectives. This is a source of failure and inefficiency of many experiments, which implies waste of research resources. A rational procedure is presented to generate the design for each particular experiment that considers the structure of the experiment appropriately. It consists of the separate formulations of the condition structure and the unit structure, and the association of these two structures by the randomization of the treatment factors and presence of the intrinsic experimental factors. This approach leads to the clear identification of the confounding of effects of the condition structure and the unit structure and the components of the experimental error and the errors that affect effects of experimental factors.***

Keywords: experimental factor; unit factor; condition structure; unit structure;experiment structure; experimental design.

1. Introduction

The foundations of experimental research were developed by R. A. Fisher between 1919 and 1933. In particular, Fisher [1] formulated the basic experiment designs and the corresponding statistical analysis procedures, such as the analysis of variance, which have become widely used in agricultural research and were soon extended to other areas. Subsequent contributions enriched the list of designs and respective statistical analysis procedures. This approach was successful in a time when computing resources were precarious and inhibited complex calculations appropriate for inferences. The dissemination of these designs and statistical analysis procedures led to their common use and misuse. Federer {2] commented: *All too often an experimenter and statistician feel their choice of an experiment and/or treatment design is limited to those appearing in tables or in the literature. The experiment should be considered as is to be conducted rather than being changed to fit a tabled design*. Federer [3] added a critique to this approach that was also common in the literature:*Many statistics books with the phrase "experimental design" or "design of experiments" in their title often have nothing on planning and almost nothing on design. Others may present a number of plans but have nothing on planning investigations*.

This is still the approach in books and texts that are used as references in teaching and research. This leads the researcher to understand that he must accommodate his experiment to a restricted set of designs, not pay attention to the planning of the experiment and, consequently, underestimate the complex interrelationship of characteristics involved in the experiment. The availability of computing resources allows the use of statistical analysis procedures appropriate for experiment with structure that expresses the relationships of characteristics consistent with the defined objectives of the research and the available experimental material.

The fundamental properties derived from randomization are the control of experimental error, in a statistical sense, and the determination of valid estimates of the uncertainties of inferences [4]. These properties require that the process of random assignment of treatments to experimental units and, in particular, the restrictions involved in this process are properly considered in the structure of the experiment, in the statistical model that express it and in the inference procedures.

Fisher [1, 5] stressed the importance of correctly considering the structure of the experiment, emphasizing that it must completely determine the statistical procedures for inferences, in particular the valid estimates of the experimental error. He distinguished two independent structures: one related to the questions to be answered by the experiment and the other associated with the classifications of the experimental units, which he called, respectively, *treatment structure* and *topographic structure*. He noted that the experiment design could be considered as the relationship between these two structures determined by randomization.

This concept of experiment design was ignored for several years and was resumed by Nelder [6, 7], and, since then, has been explored by some researchers [8, 9, 10]. However, these approaches are founded on the usual conceptual basis. In particular, they make no distinction between treatment factor and intrinsic factor, which was suggested by Cox [11]. Consequently, they ignore that intrinsic experimental factors, such as race, site and year, are "partners" of unit factors, composed of extraneous characteristics that constitute relevant classifications of the observation units.

These and many other omissions and flaws in the definitions of basic concepts in the literature originate biased inferences. For example, the importance of randomization is generally restricted to assigning treatments to their experimental units, and rare reference is made to the use of experimental techniques to control experimental error. The validity of inferences require also the appropriate use of randomization in other stages of the experiment and of experimental techniques, whenever adequate and necessary to ensure the absence of confounding of effects of treatment factors with effects of relevant extraneous characteristics.

Silva [12] discussed flaws that result from the usual definitions of basic concepts for the inferences and suggested a procedure for considering the unit effects in the statistical model and in the inferences. Silva [13, 14, 15] revised the definitions in the literature and pointed out that they were imprecise, inconsistent and ambiguous, resulting in the misunderstanding of their meanings and their incorrect application. He highlighted the consequent failures in the formulation of the experiment structure and in the specification of the statistical model to express it, and proposed a conceptual basis consistent with the actual meanings and the logical sequence of the experiment process. Silva [16] reviewed and expanded these concepts with the purpose of contributing with a rational basis for experimental research.

This paper suggests a procedure for generating the experiment structure, based on the conceptual basis proposed by Silva [16]. The structure of the experiment is derived from the separate definitions of the condition structure and the unit structure, and the association of these two structures by the randomization and presence of the experimental factors in the sample. This approach leads to the formulation of an experiment structure consistent with the research objectives, the optimization of the use of available resources and the definition of appropriate inference procedures to achieve these objectives. Special attention is given to orthogonal structures. For illustration, examples presented in Silva [16] are used.

2. Approach

The objective of the experiment is to provide inferences about effects of explanatory characteristics (experimental factors) on response characteristics in the units of a target population. These units also comprise extraneous characteristics that affect the response characteristics (Figure 1).

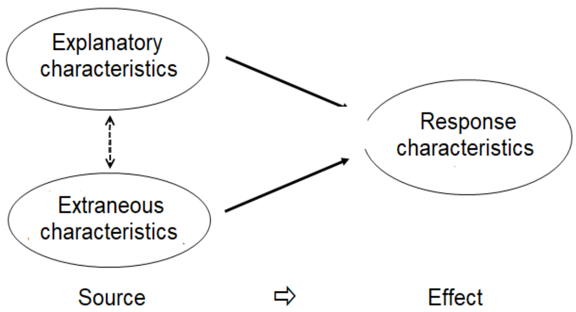

Figure 1: Diagram illustrating the causal effects of explanatory characteristics on response characteristics and the interference of extraneous characteristics

The experiment is carried out on a sample of the target population constituted during its execution. The units of the sample, as well as the units of the target population, are complex systems of characteristics that interact dynamically in space and time. This interaction causes confounding of the effects of experimental factors with effects of extraneous characteristics, which implies imprecision and bias for inferences. The planning of the experiment must consider the effects of these two sources and the relationship of these effects in order to provide the control of extraneous characteristics to achieve the desired precision and validity for the inferences.

The planning of the experiment comprises the planning of the three classes of characteristics of the sample and the relationships between the explanatory characteristics and the extraneous characteristics that have implications for the effects expressed by the response characteristics. This planning determines the **condition structure**, the **unit structure** and the **experiment structure**. The specifications of these structures establish the **experiment design**.

The condition structure must be established in line with the objectives of the experiment, while the unit structure is defined according to the available experimental material. Figure 2 shows the implications of the objectives of the experiment for the condition structure and of the available experimental material for the unit structure, and the generation of the experiment structure and experiment design by the association of those two structures.

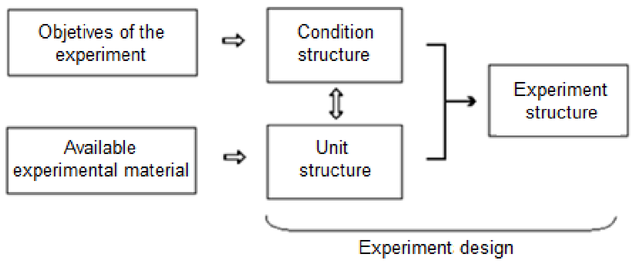

Figure 2. Diagram illustrating the generation of the experiment structure and the experiment design.

The condition structure and the unit structure are interrelated by the randomization of the levels of the treatment factors and the presence of the levels of the intrinsic factors in the experimental material. It is convenient that the planning of the condition structure and of the unit structure are carried out separately. This procedure is recommended for the experiment structure to be expressed correctly. However, the condition structure depends on the availability of experimental material and the unit structure must be appropriate for the condition structure. Thus, the definitions of these two structures are interdependent. A rational strategy for generating the experiment structure comprises the following sequence of steps:

1) Elaborate the condition structure according to the objectives of the experiment and considering the restrictions of experimental material;
2) Consider alternative unit structures for this condition structure, taking into account the available experimental material;
3) Choose, among these unit structures, the one that, associated with the condition structure, generates the experiment structure that provides the maximum information relevant to the objectives of the experiment with the minimum cost;
4) If a satisfactory unit structure is not found, reconsider the sequence of steps 1, 2 and 3.

3. Factor Relations and Representations of Factorial Structures

The symbolic notation in Bailey [10] is used in the factor relationships considered in this section and in the following ones.

An experimental factor A is a function from the set of experimental conditions $\mathcal{C}$ of size n_C to a set of n_A levels; a unit factor B is a function from the set of observation units $\mathcal{U}$ of size n_U to a set of n_B levels. Experimental factors and unit factors constitute partitions of the set $\mathcal{C}$ and the set $\mathcal{U}$, respectively. In this context, an experimental factor A is a partition of the set of conditions $\mathcal{C}$ that is significant for the objectives of the experiment. Each part of this partition is a subset of $\mathcal{C}$ corresponding to one of the n_A levels of factor A. A unit factor B is a relevant partition of the set of observation units $\mathcal{U}$. The parts of this partition are the subsets of $\mathcal{U}$corresponding to the n_B levels of factor B. Therefore, a part of a factor in $\mathcal{C}$ or $\mathcal{U}$ consists of the set of experimental conditions or observation units, respectively, corresponding to a level of that factor. The size of this part is the size of this set. A factor whose parts are of equal size is called **uniform**.

The set $\mathcal{C}$ comprises two special partitions: the partition into a single part, denoted by M_C, which does not distinguish the experimental conditions, and the partition C whose parts are the individual conditions. The partition M_C is not significant for the purposes of the experiment; therefore, it is not considered an experimental factor. Likewise, the set $\mathcal{U}$ comprises two special partitions: the partition into a single part, denoted by M_U, which does not distinguish the observation units, and the partition U whose parts are the individual observation units. The partition M_U is not considered a unit factor.

Two factors A and B (on $\mathcal{C}$ or $\mathcal{U}$) with the same parts, that is, such that every part of A is equal to some part of B and vice versa, are called **partner factors** or **equivalent factors**, which is denoted by $A \equiv B$. If A and B are inequivalent factors, B is said to be **finer** than A, or A to be **coarser** than B, which is denoted by $B \prec A$ or $A \succ B$, if the partition originated by B is finer than the partition originated by A, that is, if each part of B is contained in a part of A. Thus, for inequivalent factors A and B (on $\mathcal{C}$ or $\mathcal{U}$), $B \prec A$ means that whenever two conditions or two observation units have the same level of B, they have the same level of A. For factors A and B on the same set ($\mathcal{C}$ or $\mathcal{U}$), B is finer or equivalent to A, which is denoted by $B \preceq A$ or $A \succeq B$, if $B \prec A$ or $B \equiv A$. Thus, the set of experimental factors and the set of unit factors can be partially ordered in terms of the relations symbolized by $\prec$ and $\preceq$. In particular, if A is an experimental factor, then $C \preceq A \prec M_C$, and, if B is a unit factor, $U \preceq B \prec M_U$.

The proper definition of the relationship of each two factors in establishing the condition structure and the unit structure is of paramount importance. It is convenient that, whenever possible, factors relate in simple forms. Many experiments comprise two simple forms of relationship: nested and crossed, which are defined below. This paper considers these experiments.

The relationship of two factors A and B on the same set ($\mathcal{C}$ or $\mathcal{U}$) is **nested** (or **hierarchical**) and A and B are the nest factor and nested factors, respectively, if $B \prec A$. In this case, the levels of factor B differ between the levels of factor A and $(A \wedge B) \prec A$, but $(A \wedge B) \nprec B$. The nested relation is **balanced** if the number of levels of B is the same for all levels of A. The relationship of two factors A and B in the same set ($\mathcal{C}$ or $\mathcal{U}$) is **crossed** if $A \nprec B$ and $B \nprec A$. Then, levels of each of these factors repeat between the levels of the other factor, and $(A \wedge B) \prec A$ and $(A \wedge B) \prec B$. The crossed relation is **complete** if all levels of A appear with all levels of B, and vice versa. The condition structure and the unit structure are established by the definitions and relationships of the experimental factors and the unity factors, respectively. The forms of these relationships can be completely nested, completely crossed, or mixed, respectively if factor relationships are all nested, all crossed, or both nested and crossed.

It is convenient to classify the factors of a hierarchical or mixed factorial structure according to the hierarchy of their relationships. A factor A has a higher hierarchy than a factor B if $B \prec A$. M_C and C are the factors of the condition structure of the higher and lower hierarchy, respectively. The hierarchies of other factors are defined as follows: an experimental factor A has the second highest hierarchy if there is no coarser factor than A that is not M_C; a factor B has the third highest hierarchy if B is not equivalent to A and there is no factor coarser than B that is not A; and so forth. Similar considerations apply to factors of the unit structure.

The descriptions of the factorial structures that follow adopt the symbolic representation suggested by Wilkinson and Rogers [17] and the representation by Hasse diagrams [9, 18, 19]. The representation by Hasse diagram (**structure diagram**) is elucidative for understanding the relationships of factors and their consequences for inferences. In this paper, it is used to represent the condition structure, the unit structure and the experiment structure. It allows the identification of the experimental units of the experimental factors, the strata of the experiment where the effects of the experimental factors are located and the experimental error that affect each of these effects.

The experimental factors are represented in the condition structure as defined for the target population; their levels in this structure are the levels in the target population that are present in the sample. The extraneous characteristics comprises the unit factors that are represented in the unit structure. Experimental factors are inseparable from a subset of the extraneous characteristics. For treatment factor, it is assumed that these are irrelevant extraneous characteristics and can be supposed to behave as randomized. If an experimental factor is closely linked to disturbing extraneous characteristic, it must be considered an intrinsic factor.

In the structure diagram, each factor is represented by a dot. If a factor B is nested in a factor A and those factors have consecutive hierarchies, the point for B is below the point for A and a segment connects these two points. If two factors B and D are crossed and A is the immediately higher hierarchy factor, then B and D are represented by two separate points connected directly upwards to the point A. The points for factors Mc and C, and Mu and U are located at the upper and lower ends of the condition structure and the unit structure diagrams, respectively.

4. Formulation of the structure of the experiment

4. 1. Planning of the Condition Structure

The **condition structure** is the organization of the experimental conditions that expresses the relationships between the levels of experimental factors. It stems from the objectives of the experiment, defined by the scientific problem and hypothesis. Its planning comprises the definitions of the experimental factors, the levels of these factors and the combinations of these levels. The condition structure includes also the special factor M_C.

The condition structure can comprise one or more experimental factors. In the first case, the structure is called **unifatorial**, in the second, **multifactorial** or **factorial**. In experiments with a factorial condition structure, the factors may have different importance according to the objectives of the experiment. If the factors have different importance, they are classified as primary and secondary (or subsidiary). Primary factors are treatment factors; secondary factors can be treatment or intrinsic factors. In general, primary factors are established by the scientific hypothesis, and secondary factors are characteristics defined as experimental factors because they are expected to affect the effects of the primary factors or to obtain adequate representation of the target population.

The definition of each experimental factor and its levels for the target population and for the sample derives from the objectives of the experiment and the available resources. These definitions have decisive implications for the inferences and, in particular, for the statistical procedures for these inferences. Cox [11] distinguish the following classes of treatment factor, according to the variable chosen to express the factor and the relationship between the levels in the target population and in the sample: specific qualitative, ordered qualitative, quantitative, mixed and sampled qualitative. Specific qualitative factor can be unstructured or structured. The inference procedure for unstructured specific qualitative factor and ordered qualitative factor is the same. Thus, they can be aggregated into a single class of qualitative factor. The structured specific qualitative factor and the mixed factor can be expressed as factorial structures of qualitative and quantitative factors, as illustrated in Examples 1 and 2, below. Therefore, the five classes of treatment factors can be reduced to the following three: qualitative, quantitative and sampled qualitative. Intrinsic experimental factor can be classified as qualitative or sampled qualitative.

The levels of a qualitative factor in the target population are non-numeric, that is, they are just labels; the sample levels are the same of the target population. The levels of a quantitative factor in the target population are an interval of real numbers or a subset of it; the extremes of this interval define the scope of the inferences of interest. The sample levels are a subset of that set of levels conveniently chosen to allow the estimation of a function to express the relationship between the response variable and the treatment factor in the target population. The levels of a sampled qualitative factor in the target population are generally not all accessible or levels of a conceptual population. The sample levels are usually assumed a subset of this set chosen by a random process. However, this assumption is, in general, not tenable; consequently, the judgment of the validity of inferences for the target population is necessarily subjective.

Inferences related to qualitative and quantitative factors refer to the population means of the response variable for the levels of the factor; inferences about sampled qualitative factor refer to the population variance. For this reason, qualitative and quantitative factors are called **fixed factors,** and sampled qualitative factor, **random factor**.

The structured qualitative factor and the mixed factor originate from structured comparisons of treatments established by the objective of the experiment. As mentioned above, they can be expressed as factorial structures of qualitative and quantitative factors, as illustrated in the following two examples.

Example 1. Experiment: "Effect of using acetate on goat milk synthesis". Experimental factor: acetate - levels in the target population and in the sample: 1-prolactin 0.2 mg/kg animal weight, 2-dexamethasone 0. 2 mg/kg, 3-prolactin 0.1 mg/kg + dexamethasone 0.1 mg/kg and 4-No acetate (control).

This is a structured qualitative factor; its levels are implied by the following hierarchical structure of comparisons of groups of treatments that constitute the objective of the experiment:

1) Effect of acetate: with acetate (group 1: treatments 1, 2 and 3) versus without acetate (group 2: treatment 4),
2) Isolated acetate sources (group 3: treatments 1 and 2) versus combined acetate sources (group 4: treatment 3), and
3) Between acetate sources: prolactin (group 5: treatment 1) versus dexamethasone (group 6: treatment 2).

This structured qualitative factor can be expressed as a hierarchical factorial structure of three factors each with two levels: acetate (A), levels with (1, 2, 3) and without (4); combination of acetates (C), levels with (3) and without (1, 2); and source of acetate (S), levels prolactin (1) and dexamethasone (2). This condition structure is expressed by the symbol A/C/S and represented by the structure diagram in Figure 3.

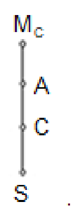

Figure 3. Structure diagram of the hierarchical factorial structure A/C/S of Example 1.

Example 2. Experiment: "Effect of biostimulant on irrigated rice production". Experimental factor: bioestimulant - levels in the sample - treatments: 1-Agrostemin 100 g/ha, 2-Agrostemin 175 g/ha, 3-Agrostemin 250 g/ha, 4-Ergostin 400 cc/ha, 5-Ergostin 500 cc/ha, 6-Ergostin 600 cc/ha and 7-without biostimulant.

Treatments 1 to 6 are the levels in the sample of two quantitative factors: Agrostemin and Ergostin, chosen from the respective intervals of levels in the target population: [100; 250] g/ha and [400; 600] cc/ha. The seven treatments constitute a mixed factor originate from the following comparisons of interest between and within treatment groups:

1) Effect of biostimulant - with (treatments 1, 2,…,6) versus without (treatment 7);
2) Fonts of biostimulant - Agrostemin (treatments 1, 2, 3) versus Ergostin (treatments 4, 5, 6);
3) Between levels of Agrostemin (treatments 1, 2, 3); and
4) Between levels of Ergostin (treatments 4, 5, 6).

This factor can be expressed as a hierarchical factorial structure of the factors: biostimulant (B) - levels with (1,2,…, 6) and without (7); source of biostimulant (S) - levels Agrostemin (1, 2, 3) and Ergostin (4, 5, 6); and dose (D) - levels in the target population: intervals [100, 250] g/ha and [400, 600] cc/ha for Agrostemin and Ergostin, respectively, and levels in the sample: 100, 175, 250 g/ha for Agrostemin and 400, 500, 600 cc/ha for Ergostin. This condition structure is expressed by the symbol B/S/D and represented by the diagram in Figure 4.

Figure 4. Structure diagram of the hierarchical factorial structure B/S/D of Example 2.

The family of factorial condition structures is very broad and comprises apparent (false) factorial and extended factorial structures that can be expressed as real factorial structures. Example 3 illustrates an apparent factorial structure that is, in fact, an extended factorial structure that can be expressed as a mixed factorial structure.

Example 3: Experiment: "Fertilizing the soil with nitrogen for irrigated rice cultivation". Experimental factors: nitrogen, with levels in the target population comprising the interval [0; 120] kg/ha and three levels in the sample: n_1-0, n_2-60 and n_3-120 kg/ha; and time of application, with two levels in the target population and in the sample: t_1-planting and t_2-coverage at 30 days. The treatments are shown in Table 1.

Table 1: Treatments of the nitrogen fertilization experiment, Example 3.

Treatment	N	Time
1 - n_1t_1	0	planting
2 - n_1t_2	0	coverage
3 – n_2t_1	60	planting
4 – n_2t_2	60	coverage
5 – n_3t_1	120	planting
6 – n_3t_2	120	coverage

The combinations of levels of n and time n_1t_1 and n_1t_2 (Table 1) are not distinguished and constitute the same treatment - no nitrogen. Therefore, the 3x2 = 6 combinations of the levels of n and time constitute, in fact, five treatments of a crossed structure of two factors - nitrogen with two levels: 60 and 120 kg/ha and time with two levels: planting and coverage, extended by an additional treatment: no nitrogen. The six treatments can be expressed as a mixed factorial structure of three factors: fertilization (F) with two levels - without and with, nitrogen (N) with two levels - 60 and 120 kg/ha, and time (T) with two levels - planting and coverage. Factors N and T are crossed and both are nested in factor F, constituting a mixed factorial structure symbolized by F/(N∗T) and represented by the diagram in Figure 5.

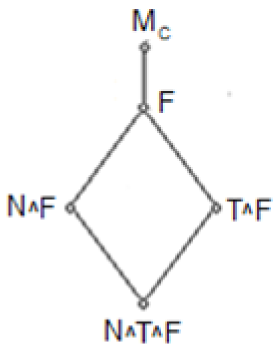

Figure 5: Structure diagram of the apparent factorial structure in Example 3 expressed as a mixed factorial structure: F/(N∗T).

4. 2 Planning of the Unit Structure

The **unit structure** is the organization of the observation units that expresses the relationships between the levels of the unit factors. It stems from the classifications of the extraneous characteristics determined by the observation units, the experimental units and the local control. Its planning comprises the definitions of the unit factors, the levels of these factors and the combinations of these levels. The unit structure includes also the special factor M_U.

The unit structure should be suitable for the condition structure. Like the condition structure, it can comprise one or more factors. In case of more than one factor, relations of factors can be nested or crossed, and the unit structure can be completely nested, completely crossed or mixed.

Unit factors are random factors and it is assumed that there is no interaction between experimental factor and unit factor. These assumptions are necessary for inferences for the units of the population represented by the sample. If a unit factor comprises a non-random component or interacts with an experimental factor, that component must be defined as an intrinsic experimental factor, equivalent to that unit factor.

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For example, if at the place where a wheat experiment is carried out the terrain has a slope, the local control must form blocks according to level ranges. In this case, if the level range can affect the effects of treatments, the level range should be considered an intrinsic experimental factor, equivalent to the block factor.

4. 3. Planning of the Experiment Structure

The **experiment structure** is the organization of the relationship between the factors of the condition structure and the factors of the unit structure, resulting from the association of these two structures by randomization of the levels of the treatment factors the presence of the levels of the intrinsic factors. The generation of the experiment structure and the experiment design is illustrated in Figure 6.

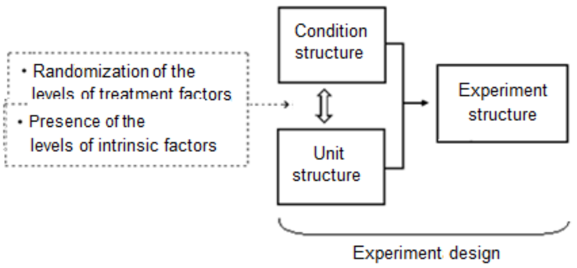

Figure 6. Generation of the experiment structure and the experiment design.

In the structure of the experiment, there is an association between the unit factors and the experimental factors. This structure comprises factor relationships in addition to crossed and nested relationships present in the condition structure and the unit structure. These relationships are considered in this Section.

The unit factors identify strata of the experiment, which correspond to strata of the experimental error. Each of these strata may or may not include an experimental factor. In each stratum where an experimental factor is located, there is a correspondence between the levels of that factor and the levels of the unit factor. The levels of a unit factor associated with an experimental factor are the **experimental units** of this experimental factor. The **experimental error that affects the effect of an experimental factor** is the variation of the extraneous characteristics between the experimental units of this factor.

In general, the unit factor is coarser or equivalent to the experimental factor, that is, if B is a unit factor and A is an experimental factor, $B \succeq A$. Thus, the correspondence between A and B can be of two forms:

a) To each level of A corresponds more than one level of B, which is symbolized by $B \succ A$. In this case, there is more than one experimental unit for levels of A. Experimental units with different levels of A are different, but for a same level of A there is more than one experimental unit. This implies that effects of the experimental factor A are completely confounded with experimental error (effect of unit factor B), but this experimental error is partially confounded with effects of A. In this situation, with appropriate experimental control, particularly randomization, A is a treatment factor. Randomization depends on the unit structure:

i) if B is the only unit factor, randomization is performed by assigning treatments (levels of A) to experimental units (levels of B) without any restriction;

ii) if B is preceded by a unit factor D, randomization is performed by assigning treatments to levels of B within the levels of D.

In these two circumstances, a procedure for inferences about effects of treatment factor A is to compare a source of variation of the observed values of the response variable that includes the effects of A with another source that expresses the same effects, except for these effects of treatment factor A. If the common component of these two sources is random and the first source proves to be greater than the second due to a difference that cannot be considered random, this superiority is attributed to the existence of effect of factor A. In this circumstance, the common component of the two sources of variation provides a valid estimate of the error that affects the effects of treatment factor A. This common component, which does not include effects of A, comprises experimental error. If this stratum also contains random experimental factors, this component may include also effects of these factors. In case i), the experimental error comprises the variation between experimental units with same treatment, which means that it comes exclusively from the stratum B. In case ii), there are two possibilities: if the size of each level of the unit factor D equals the number of treatments and each level of D includes all treatments, the experimental error comprises only the variation among experimental units within the levels of D; if the size of the levels of D is less than the number of treatments, it includes also experimental error between these levels, that is, from stratum D.

b) To each level of experimental factor A corresponds a level of unit factor B, that is, there is a one-to-one correspondence between the levels of these two factors, which means that A and B are equivalent factors and is symbolized by $B \equiv A$. Therefore, the effects of A and B are completely confounded. In this situation, the stratum corresponding to unit factor B does not have pure experimental error as one of its components. The equivalence of an experimental factor and a unit factor occurs in the following circumstances:

i) The experimental factor A is an intrinsic factor. Then, valid inferences about the effects of this experimental factor cannot be derived.

ii) The experimental factor A is a treatment factor with a single repetition of each of its levels. In this situation, inferences about the treatment factor cannot be derived, except in experiments with factorial condition structures in which high level interactions can be assumed non-existent so that their component can be attributed to experimental error. This assumption can be tenable under stable environmental and management conditions, as can occur in industry.

Desirable properties of inferences about treatment factors demand that the plan of the experiment ensures that the structure of the experiment is consistent with its objectives and the following requirements are met [11, 20]:

- Estimation of the components of the experimental error that affect the relevant effects of treatment factors;
- Precision - sensitivity to detect important differences of treatment effects;
- Validity - unbiasedness of inferences:
- External validity - unbiasedness of the sampling error,
- Internal validity - unbiasedness of the experimental error;
- Implicity, economy of resources, feasibility;
- Manifestation of the real effects of the treatments; and
- Provision of statistical inference procedures and measures to assess the degree of uncertainty of these inferences.

These requirements demand some properties of the experiment design. These properties are the following, usually called **principlesof the experiment design**: repetition, local control, randomization, orthogonality, balance, confounding and efficiency. Repetition, local control and randomization where considered by Silva [16]. Here, special attention is paid to orthogonality (Section 5). With orthogonality, effect of an experimental factor is confined to one stratum of the experiment. Orthogonality is a property satisfied by the treatment factor A and unit factor B in situation a-i), and can be applied in a-ii when the number of treatments equals the number of levels of the unit factor B within each level of unit factor D. In the absence of orthogonality, the effect of an experimental factor can be expanded to a higher level stratum. For experiments in which orthogonality is not convenient or appropriate, the properties of balance and confounding are useful. Balance is appropriate in situation a-ii when the number of levels of treatment factor A is larger than the number of levels of the unit factor B within each level of D. Confounding is a property applicable in situation b-i under special conditions. These properties are not considered here.

5. Orthogonality and Orthogonal Structures

Orthogonality is an important property of the experiment design, since it allows the derivation of inferences about each effect of treatment factor separately and independently of the effects of other experimental factors and of unit factors. Orthogonality is considered here because it is also very convenient for understanding the generation of structures, to derive statistics for inferences and to make these inferences. Particularly, it allows the ease calculation of degrees of freedom, sums of squares and other statistics for inferences about treatment factors by using structure diagrams.

5. 1 Orthogonality of Factors

Factors A and B on the same set (($\mathcal{C}$ or $\mathcal{U}$) are **orthogonal** to each other if and only if:

- A and B have nested (hierarchical) relation; or
- A and B have crossed relation and the intersections of the parts of A and B have sizes proportional to the product of the sizes of the corresponding parts of A and B; that is, $n_{ab} = (n_a n_b)/n$, for all levels of factor $A \wedge B$, where n_a, n_b and n_{ab} are the sizes of the a-th part of A, the b-th part of B and of their intersection, respectively.

In Example 1, the factors acetate (A), combination of acetates (C) and source of acetate (S) comprise a chain of nested factors; so, they are mutually orthogonal. For the same reason, the factors biostimulant (B), source (S) and dose (D) in Example 2 are mutually orthogonal. In Example 3, the factors nitrogen (N) and time (T) are crossed and both nested in the factor fertilization (F); therefore, both factors N and T are orthogonal to factor F. The orthogonality of N and T depends on the sizes of the parts of the factor $N \wedge T$. Figure 7 illustrates three situations that satisfy the requirement for orthogonality of N and T.

T	N	
	60	120
Planting	4	4
Coverage	4	4

T	N	
	60	120
Planting	4	2
Coverage	4	2

T	N	
	60	120
Planting	4	2
Coverage	2	1

Figure 7: Three different proportions of sizes of the parts of factor $N \wedge T$ that satisfy the condition for orthogonality of factors N and T

5. 2. Orthogonality of Structures

Requirements for the orthogonality of the condition, unit and experiment structures:

Condition structure:
a) The special factor M_C belongs to the structure,
b) experimental factors are mutually orthogonal.

Unit structure:
a) The special factor M_U belongs to the structure,
b) unit factors are mutually orthogonal,
c) unit factors are uniform.

Experiment structure:

a) The M factor, with a single level, which results from the association of special factors M_C and M_U, belongs to the structure,
b) the condition structure is orthogonal,
c) the unit structure is orthogonal,
d) the experimental factors remain mutually orthogonal,
e) the experimental factors are orthogonal to the unit factors.

6. Illustration

To benefit understanding, the examples in this Section are restricted to orthogonal structures. They illustrate condition structures, unit structures and resulting experiment structures, using examples in Silva [16].

Example 4. Experiment: "Effect of energy diet on the body development of male lambs between weaning and slaughter", considering the response variables live weight, warm carcass weight and carcass yield [16, Example 1].

The experiment considers diet in the interval [2. 4; 3. 2] Mcal/kg DM of metabolizable energy in the target population, and the three levels 2. 4, 2. 8 and 3. 2 in the sample. It is carry out on a farm with 30 male animals of a race aged close to 70 days. These animals are housed in 15 pens for two animals equipped with drinkers and feeders. As the characteristics of the environment and the weaning dates of the animals are heterogeneous, the 15 pens and the 30 animals are classified into five blocks of three nearby pens and six animals with weaning at close dates. In each block, the three pens with two animals are randomly assigned to the three diets, separately and independently for each block. The response variables are measured in each animal at slaughter. The experimental procedure defines diet as a treatment factor, pen as its experimental unit and the animal as the observation unit of the response variables.

The set of experimental conditions $\mathcal{C}$ consists of the three levels of the single experimental factor diet (D), which is a fixed factor. Thus, the set of experimental factors is {D}; the condition structure is expressed by the symbol D and represented by diagram in Figure 8a. This condition structure is orthogonal.

The set of observation units $\mathcal{U}$ contains 30 units, which are the levels of the unit factor animal (U); the animals are grouped into pairs, which are the levels of the unit factor pen (P); and the pens are classified into blocks, which form the unit factor block (B). Thus, the set of unit factors is {U, P, B}. These factors are uniform and constitute a nested structure, symbolized by B/P/U and represented by diagram in Figure 8b. Therefore, the unit structure is orthogonal.

The condition structure D and the unit structure B/P/U are associated by the randomization of the levels of the unit factor P to the levels of the treatment factor D within each level of unit factor B. The double-headed arrow with a dashed line in Figure 8a,b identifies P as the unit factor whose levels are the experimental units of the treatment factor D. This association generates the strata of the experiment B, P≥D and U (Figure 8c), where P≥D symbolizes that the unit factor P is partially confounded with treatment factor D. The strata B and U do not include experimental factor. The treatment factor D is located in the stratum P≥D, and is crossed with the unit factor B, and both nest factor B∧D≡P, which nests the unit factor U. Thus, the structure of the experiment comprises the factors B, D, P≡B∧D and U. It is represented by the symbol (B∗D)/U and diagram in Figure 8d.

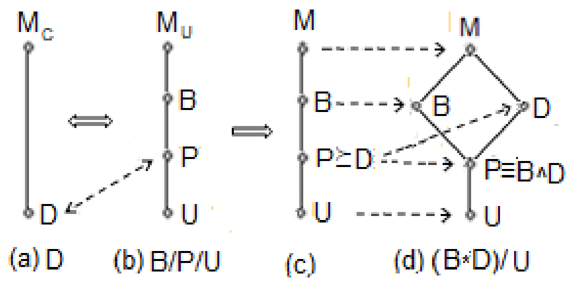

Figure 8: Diagram of the generation of the experiment structure (B∗D)/U: (a) condition structure, (b) unit structure, (c) strata of the experiment and (d) experiment structure

In addition to the condition structure and the unit structure being both orthogonal, it can be shown that in the structure of the experiment the experimental factor remain orthogonal and is orthogonal to the unit factors. Therefore, the experiment structure is orthogonal. All information for inferences about effects of the diet factor come from the stratum P≥D. In this stratum, to different diets correspond different experimental units (pens), but to the same diet correspond five experimental units. This implies that the variation between experimental units of the observed values of the response variable due to effects of diets is completely confounded with the experimental error, and the experimental error is partially confounded with diet effects. The fraction of this experimental error within blocks not confounded with diet effects is the variation between pens, excluding variation between diets. This variation also expresses the interaction between diet and block. However, the absence of interaction between experimental factor and unit factor is a general assumption of the structure of experiment. With this assumption, randomization and use of adequate experimental control, the variation between pens, excluding variation between blocks and diets, provides a valid estimate of the variance of the error that affects the effects of diets.

Example 5. Experiment: "Control of giberela in wheat crops in the State of Rio Grande do Sul" [16, Example 2], considering one place in one year.

The experiment is carried out in 48 plots of a terrain heterogeneous regarding to soil characteristics that are classified into four blocks of 12 homogeneous plots. The 12 plots of each block are randomly assigned to the 12 combinations of the levels of the fungicide and cultivar factors, separately and independently for each block. The response variables grain yield, hectoliter weight, thousand grain weight, number of spikelet, number of infected spikelet, incidence and severity of giberela are measured in each plot. Thus, fungicide∧cultivar and, by consequence, fungicide and cultivar are treatment factors; plot is the common experimental unit of these treatment factors and is the observation unit of the response variables.

The set of experimental conditions $\mathcal{C}$ comprises the twelve combinations of the four levels of the factor fungicide (F) with the three levels of the factor cultivar (C). Therefore, the treatment factors F and C are crossed and nest factor F∧C. F and C are both fixed factors. Therefore, the set of

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experimental factors is {F, C, F∧C}, which can be verified to constitute an orthogonal condition structure, expressed by the symbol F∗C and represented by diagram in Figure 9a.

The set of observation units $\mathcal{U}$ has 48 units, which are the levels of the unit factor plot (U). These plots are classified into blocks, which constitute the unit factor block (B). Therefore, the set of unit factors is {U, B}. The two unit factors U and B are uniform and have a nested relation. Therefore, the unit structure is orthogonal, and is symbolized by B/U and represented by diagram in Figure 9b.

The structure of the experiment is established associating the condition structure F∗C and the unit structure B/U by the randomization of the levels of the unit factor U to the levels of the treatment factor F∧C within each level of the unit factor B. Figure 9a,b identifies U as the unit factor whose levels are the experimental units of the treatment factor F∧C, and, consequently, of treatment factors F and C. This association generates the strata of the experiment B and U≥(F∧C) (Figure 9c). The treatment factors F, C and F∧C are located in the stratum U≥(F∧C). The F and C factors are crossed and nest the F∧C factor. The treatment factors F, C and F∧C are also crossed with the unit factor B. Thus, the experiment structure comprises factors B, F, C, F∧C and the combinations of the unit factor B with the treatment factors F, C and F∧C, that is, B∧F, B∧C and B∧F∧C. As these factors originate interactions of a unit factor with experimental factors, which must be considered non-existent, the factors B∧F, B∧C and B∧F∧Care aggregated into factor F∧C∧B≡U, whose effect is experimental error. This experiment structure is represented by the symbol F∗C∗B and diagram in Figure 9d.

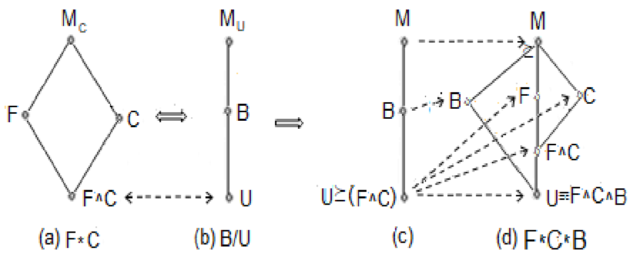

Figure 9: Diagram of the generation of the experiment structure F∗C∗B: (a) condition structure, (b) unit structure, (c) strata of the experiment and (d) experiment structure.

In this experiment structure, the experimental factors are mutually orthogonal and are orthogonal to the unit factors. As the condition structure and the unit structure are both orthogonal, the experiment structure is orthogonal. All information for inferences about effects of treatment factors F, C and F∧C comes from the stratum U≥(F∧C). In this stratum, to different levels of each of these factors correspond different experimental units (plots), but to a same level of F, C and F∧C correspond 12, 16 and 4 experimental units, respectively. This implies that the variation between experimental units due to effects of these treatment factors is completely confounded with the experimental error, but the experimental error is partially confounded with effects of F, C and F∧C. The fraction of this experimentalerror within blocks not confounded with effects of fungicide, cultivar and fungicide∧cultivar is the variation between plots, excluding variation between levels of these treatment factors. This variation expresses also the interactions of fungicide, cultivar and fungicide∧cultivar with block, which are assumed non-existent. Therefore, due to randomization and with appropriate experiment control, this variation provides a valid estimate of the variance of the error that affects the effects of the treatment factors fungicide and cultivar.

Example 6: Experiment: "Effect of energy diet and growth stimulant on the body development of male lambs between weaning and slaughter"[16, Example 1c].

Suppose a change in the objectives of the experiment in Example 4 that considers a growth stimulant with two levels as another experimental factor. Now, the experiment is carried out with a group of 30 animals in a facility with 15 pairs of pens for one animal, each pair equipped with common feeder and drinker. As the 30 animals and the 15 pairs of pens are heterogeneous, they are classified into five homogeneous blocks of three pairs of pens, each pen with one animal. Then, the pairs of pens of each block are assigned at random to the three diets and the two pens of each pair are randomized to the two levels of the stimulant factor. The response variables live weight, warm carcass weight and carcass yield are measured in each animal (pen). With this procedure, diet and stimulant are treatment factors, their experimental units are the pair of pens and the pen, respectively, and the observation unit of the response variables is the pen.

The set of experimental conditions $\mathcal{C}$ contains the six combinations of the three levels of the factor diet (D) with the two levels of the factor stimulant (S), both fixed factors. Therefore, the treatment factors D and S are crossed and nest factor D∧S, and the set of experimental factors is {D, S, D∧S}. The condition structure is represented by the symbol D∗S and diagram in Figure10a. It can be verified that it is orthogonal.

The set of observation units $\mathcal{U}$ contains 30 animals, which are the levels of the unit factor pen (U). The pens constitute pairs that are the levels of the unit factor pair of pens (P), and the pair of pens are classified into blocks to form the unit factor block (B). Therefore, the set of unit factors is {U, P, B}. The three unit factors U, P and B are uniform and constitute a nested structure, symbolized by B/P/U and represented by diagram in Figure10b. Therefore, the unit structure is orthogonal.

The association between the unit structure B/P/U and the condition structure D∗S establishes correspondence between the unit factors P and U with the treatment factors D and S, defines the the levels of the unit factors P and U as the experimental units of the treatment factors D, and S and D∧S, respectively (Figure 10a,b), and constitute the strata of the experiment B,P≥D and U≥S (Figure 10 c). In the structure of the experiment, factors B, D and S are crossed; factor B∧D is nested in factors B and D, factor D∧S is nested in D and S, B∧S is nested in B and S and B∧D∧S is nested in these factors. Thus, the experiment structure comprises the factors B, D, S, P≡B∧D, D∧S and the combinations of S and D∧S with B, that is, B∧S and B∧D∧S. As the effects of these last two factors are interactions of experimental factor with unit factor, which are assumed non-existent, they are aggregated into factor B∧D∧S≡U, whose effect is experimental error. This experiment structure is represent by the symbol B∗D∗S and diagram in Figure 10d. It can be shown to satisfy the requirements for orthogonality.

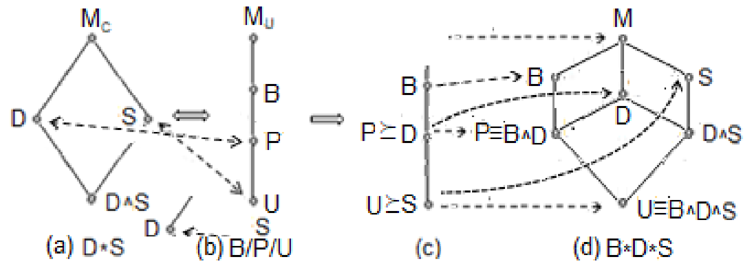

Figure 10: Diagram of the generation of the experiment structure B∗D∗S: (a) condition structure, (b) unit structure, (c) strata of the experiment and (d) experiment structure.

All information for inferences about the effects of the treatment factor diet comes from the stratum P≥D, and for inferences about the effects of the treatment factors stimulant and diet∧stimulant from stratum U≥S. The considerations regarding inferences about the diet factor are as described in Example 5, changing the meanings of the symbols of the unit factors as follows: U - pen, P - pair of pens and B - block. In the stratum U≥S, to different levels of the factors stimulant and diet∧stimulant correspond different experimental units (pens), but for each of these factors for a same level correspond 15 and 5 experimental units, respectively. This implies that the variation between experimental units due to effects of stimulant and diet∧stimulant is completely confounded with experimental error, but this experimental error is partially confounded with effects of these treatment factors. The fraction of this experimentalerror between pairs of pens not confounded with effects of stimulant and diet∧stimulant is the variation between pens, excluding variation between levels of these treatment factors. This variation express also the interactions of stimulant and diet∧stimulant with block, which are assumed non-existent. Because of randomization and with appropriate experimental control, this variation provides a valid estimate of the variance of the error that affects the effects of stimulant and diet∧stimulant.

Example 7: Experiment: "Effect of energy diet on the body development of lambs between weaning and slaughter" (16, Example 1a].

Suppose the objectives of the experiment in Example 6 are changed to consider race as an experimental factor with two levels, instead of growth stimulant. The three pairs of pens of each block are randomized to the three diets and the two pens of each pairare assigned at random to two animals, one of each race. As in Example 6, the response variables live weight, warm carcass weight and carcass yieldare measured in each animal, diet is a treatment factor, its experimental unit is the pair of pens, and the observation unit of the response variables is the pen. However, the experimental factor race may be a treatment factor or an intrinsic factor, depending on the extraneous characteristics of the animal. If these extraneous characteristics are irrelevant so that the differences between animals of the two races are essentially due to race, one can consider race as a treatment factor. This can occur if animals of both races come from the same herd and proper experimental control is used. In this case, the experiment structure is as defined in Example 6 with stimulant substituted for race, and all considerations made there are valid here. However, different races are usually raised in separated herds. This implies that the differences between the animals of the two races are also due to extraneous characteristics of the environment and management prior to the experimental period, which are not randomized to races. If these characteristics are relevant, race should be considered an intrinsic factor. This situation is considered now.

The set of experimental condition $\mathcal{C}$ contains the six combinations of the three levels of the treatment factor diet (D) with the two levels of the intrinsic factor race (R). Diet and race are both fixed factors. The set of experimental factors is {D, R, D∧R} and the condition structure is crossed, which is symbolized by D∗R and represented by the diagram in Figure 11a. It can be verified that it is orthogonal.

The set of observation units $\mathcal{U}$ comprises 30 animals, as in Example 6. Also, as in that Example, the set of unit factors includes the factors U: pen, P: pair of pens and B: block; but now has an additional factor composed by the extraneous characteristics of the animal whose effects are completely confounded with effects of the intrinsic factor race (R). This unit factor, which has two levels, is called herd and denoted by H. Each level of this factor is the set of the extraneous characteristics of the animal of a race. Thus, the experimental factor R is equivalent to the unit factor H: R≡H. This implies that the experimental unit of the intrinsic factor race is a herd and, therefore, there is only one experimental unit for each level of this experimental factor. The set of unit factors is {P, B, H, U}. The unit structure is represented by the symbol ((B/P)∗H)/U and diagram in Figure 11b. It can be shown that it is orthogonal.

The association of the unit factors P and H with the experimental factors D and R, respectively, defines the structure of the experiment and generates the strata of the experiment B, P⪰D, H≡R and U. In this structure, factors B, D and R≡H are crossed, P is nested in B and D, D∧(H=R) is nested in D and H≡R, B∧(H=R) is nested in B and H≡R and B∧D∧(H≡R) is nested in these factors. It comprises factors B, D, P≡B∧D, H≡R, D∧(H=R), B∧(H≡R) and B∧D∧(H≡R). As the effects of the last two factors are interactions of unit factor and experimental factor, assumed to be non-existent, they are aggregated into factor B∧D∧(H≡R)≡U. This experiment structure is represented by the symbol B∗D∗(H≡R) and diagram in Figure 11d, and can be shown to be orthogonal.

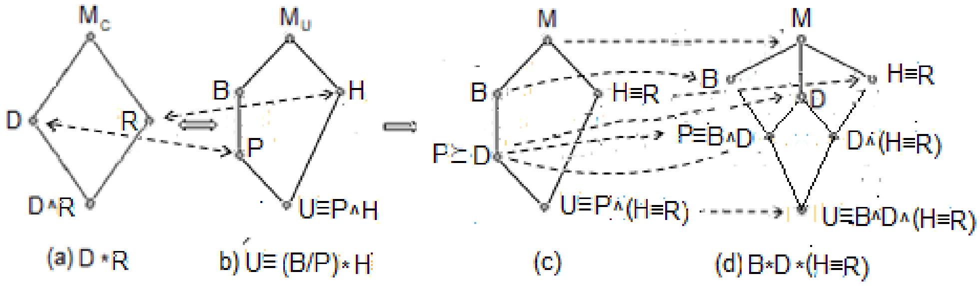

Figure 11: Diagram of the generation of the experiment structure B∗D∗(H≡R): (a) condition structure, (b) unit structure, (c) strata of the experiment and (d) experiment structure

Figure 11a,b,c identifies P and H as the unit factors whose levels are the experimental units of the experimental factors D and R, respectively, and P⪰D and, H≡R as the strata of the experiment where these experimental factors are located. Considerations about the error that affects effects of the diet factor are the same as in Example 6. In the stratum H≡R the effects of the experimental factor R are completely confounded with effects of the unit factor H. Therefore, this experiment structure does not provide valid estimate of the variancenof the error that affects effects of the experimental factor race and, therefore, does not allow inferences about these effects. For a similar reason, inferences about the interaction between race and diet are also not allowed. .

Example 8: Experiment: "Control of the incidence of giberela in wheat crops in the State of Rio Grande do Sul" (16, Example 2], considering one year of the experiment.

Suppose the experiment in Example 5 is carried out in four locations of the wheat-growing region under consideration. The characteristics of the experiment in each location are as described in that Example. However, now a new experimental factor is considered: site. This is an intrinsic factor, and an equivalent unit factor should be considered, which is named place. The experimental factor site is composed by the permanent characteristics of the locations where the experiment is carried out, mainly related to soil and weather. The unit factor place comprises the extraneous characteristics that eventually occur in the year of execution of the experiment, such as atypical occurrences of climatic events, insects, diseases and predators, and failures of experimental techniques.

The set of experimental conditions $\mathcal{C}$ contains the 48 combinations of the four levels of the factor fungicide (F), the three of the factor cultivar (C) and the four of the factor site (S). Therefore, the set of experimental factors is {F, C, F∧C, S, F∧S, C∧S, F∧C∧S}, where F and C are fixed factors and S is random factor. It can be shown that this set of factors constitute a crossed orthogonal condition structure represented by the symbol F∗C∗S and diagram in Figure 12a.

The set of observation units $\mathcal{U}$ contains 192 units (48 in each place) that are the levels of the unit factor plot (U), which are grouped into levels of the factor block (B) in each level of the factor place (P). Thus, the set of unit factors is {U, B, P}. The unit factors U, B and P are uniform and constitute a nested structure, symbolized by P/B/U and represented in Figure 12b; therefore, it is orthogonal.

The association of the unit factor P with the intrinsic factor S generates the stratum of the experiment P≡S, and, in each place, of the unit factor U with the treatment factor F∧C gives rise to the stratum U⪰(F∧C). In each place, factors F and C are crossed and both nest F∧C. These three factors are crossed with factors S and B. The resulting experiment structure comprises the factors F, C, F∧C, P≡S, F∧(P≡S), C∧(P≡S), F∧C∧(P≡S), B(P≡S), F∧B(P≡S), C∧B(P≡S) and F∧C∧B(P≡S). The last three factorscan be aggregate into factor F∧C∧B(P≡S)≡U, which originates experimental error. This experiment structure is symbolized by F∗C∗((P≡S)/B) and diagram in Figure 12d. Besides the condition structure and the unit structure being both orthogonal, the experimental factors remain mutually orthogonal in the structure of the experimentand are orthogonal to the unit factors. Therefore, the experiment structure is orthogonal.

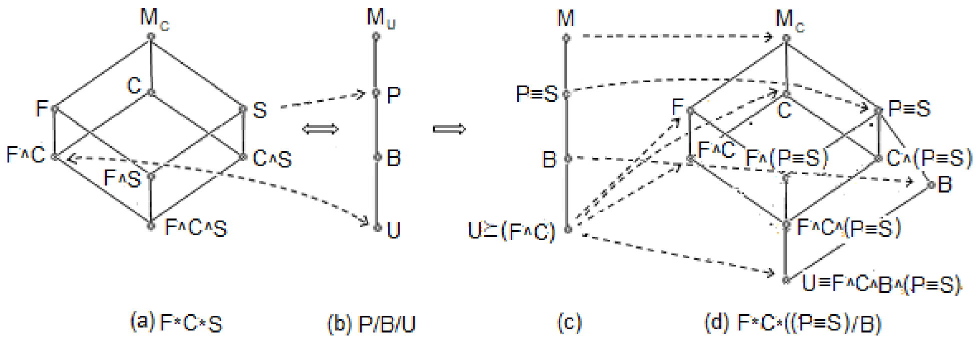

Figure 12: Diagram of the generation of the experiment structure F∗C∗((S≡P)/B): (a) condition structure, (b) unit structure, (c) strata of the experiment and (d) experiment structure.

As in Example 5, the experimental units of the treatment factors F, C and F∧C are the levels of the unit factor U (plot), and thesetreatment factors are located in the stratum U⪰(F∧C). This stratum also includes the random experimental factors F∧(P≡S), C∧(P≡S) and F∧C∧(P≡S), whose effects affect the effects of the treatment factors F, C and F∧C, and B∧F, B∧C and B∧F∧C. Therefore, the error that affects effects of these treatment factors include experimental error and their interactions with P≡S and with B. In the stratum U⪰(F∧C), to different levels of F, C and F∧C correspond different plots, but to a same level of these treatment factors correspond 12, 16 and 4 plots, respectively. This implies that the variation between plots due to effects of F, C and F∧C is completely confounded with that error, which is partially confounded with effects of those treatment factors. The fraction of this error within blocks and places not confounded with effects of fungicide, cultivar and fungicide∧cultivar is the variation between plots, excluding variation between levels of fungicide, cultivar and fungicide∧cultivar. This variation expresses experimental error within blocks, the interactions of the factors F, C and F∧C with P≡S and also the interactions of these treatment factors with block, which are assumed non-existent. Supposing the use of adequate experimental control, particularly randomization, this variation provides a valid estimate of the variance of the error that affects effects of the treatment factors fungicide and cultivar. The experimental factor site (S) is located in the strata P≡S, where its effect is completely confounded with the effect of the unit factor place (P). This implies that this experiment structure does not provide a valid estimate of the variance of the error that affects the effects of the site experimental factor and does not allow valid inferences about these effects and their interactions with fungicide, cultivar and fungicide∧cultivar.

7. Conclusions

- The clear separation between experimental factors and unit factors, particularly the identification of intrinsic experimental factors, is essential to distinguish the characteristics object of inferences from those that can affect them substantially. The subsequent separate formulations of the condition structure and the unit structure, and the association of these two structures consistent with the objectives of the experiment allow the construction of the appropriate experiment structure to achieve these objectives.
- Crossed and nested relationships determine a partial order of factors in the experiment structure, which can be represented by a structure diagram. This is a practical tool to identify the strata of the experiment where the experimental factors are located and the experimental units of these factors. It also allows identifying the confounding of effects of experimental and unit factors, and the errors that affect effects of experimental factors. This information makes it possible to outline the path to inferences about effects of treatment factors and clarifies the impossibility of valid inferences about effects of intrinsic factors.

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