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Experimental research

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Abstract

Explanatory research has the goal of verifying a scientific hypothesis regarding the causal relationship between a set of response characteristics and a set of explanatory characteristics of a target population. The experiment is the explanatory research method par excellence. In the experiment, the researcher chooses the levels of one or more explanatory characteristics, associates these levels with the units of the sample by a random process and controls the presence of extraneous characteristics. Thus, the confounding of the effects of extraneous characteristics with the effects of the explanatory characteristics is reduced and its bias is avoided. This sample control has consequences relevant to inferences. This paper outlines the evolution of experimental research, makes a characterization of the experiment, describes the experimental research process and highlights the importance of considering statistical methodology in this process.

Keywords: History of experimental research; Controlled experiment; Randomization; Experimental design; Experiment process; Experiment protocol; Experimental statistics.

1 Introduction

The experiment is the explanatory research method in which the researcher intervenes in the sample deliberately imposing the levels of one or more explanatory characteristics on its units with the purpose of generating inferences about the causal effects of these characteristics on response characteristic. These are extrinsic or treatment characteristics. The experiment can also include intrinsic characteristics, which manifest themselves in the sample, without control or with limited control of the researcher. Levels of extrinsic characteristics are stimuli, such as diets administered to animals or fungicides applied to plants.

This article reviews experimental research to introduce this research method for researchers. This review is a sequence of Silva [1, 2, 3]. The conceptual basis of scientific research and the characterization of the explanatory research, particularly of the experiment, was considered in Silva [2, 3]. This paper focus on topics of experimental research that seem most important. A summary of the evolution of the experimental method is the theme of section 2. Section 3 presents the concepts of experiment in several contexts and corresponding classifications, and points out that this paper considers the comparative randomized experiment. Section 4 illustrates experimental research and the relevant concepts defined in [8], and characterizes experiment as the explanatory research method that provides greater validity for inferences. Section 5 discusses the process of experimental research, considering the major stages of the experiment. The experiment process is detailed in section 6 by a reference list that summarizes the steps and care that must be taken when running an experiment. Section 7 highlights the importance of the written documentation of the experiment, particularly the experiment plan. Finally, section 8 addresses the role of Statistics in experimental research and establishes a concept of Experimental Statistics.

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This review is based primarily on contributions from Fisher [4, 5], Yates & Cochran [6], Cochran [7, 10, 11], Silva [8, 22, 23, 24, 25, 26], Bernard [9], Cox [12, 13], Christensen [14], Federer [15], Heath [16], Kempthorne [17], Kish [18, 19], Ostle [20], Selwyn [21], Urquhart [27], Wilson [28].

2 Origin and Evolution of Experimental Research - Brief History

The current concept of experiment as a method of scientific research is recent. The experimental method evolved gradually. The milestones of this evolution and the most relevant contributions are summarized below.

The origin of experimental research is often attributed to the English philosopher Francis Bacon (1561-1626) in the 17th century. However, the experimental method dates back at least four centuries before Christ, when Aristotle (384-322 b.C.) made several discoveries concerning the natural world. Based on experiments, axioms and philosophical arguments, Aristotle concluded, for example, that the acceleration of a free-fall body depends on its mass, and that the earth should be a sphere, since the sphere is the most "perfect" solid. In the 2nd century, the astronomer and mathematician Claudius Ptolemy (90-168 a.C.) of Alexandria developed a theory of the universe from the ideas of Aristotle. Through geographical arguments, Ptolemy stated that the earth was a sphere and, being immobile, it should be the center of the universe, because, if it were not, the lighter air would be left behind, because of its slower acceleration.

These theories were accepted without controversy and remained so until the Middle Age. The percussor of the new era of scientific inquiry was the English philosopher Roger Bacon (1220-1292), who made mathematics and the experimental method the basis of natural history. The term "experimental science" was popularized by his work. According to him, man acquires knowledge through reasoning and experience, but without experience he cannot be sure.

In the 16th century, polish astronomer Nicholas Copernicus (1473-1543) revived the theories of the solar system originally formulated by the Greek philosopher and mathematician Pythagoras (580-497 b.C.) six centuries before Christ. Copernicus maintained that the sun should be the center of the universe. However, his work was published only after his death.

The English physician and physicist William Gilbert (1544-1603), particularly interested in magnetism, was one of the first experimenters. He systematically subjected the existing knowledge and its conjectures to experimental testing. In addition, he realized the need for repetition, in the case of expectation of variation of the result of an experiment. At that same time, Francis Bacon (1561-1626) maintained that the starting point of all science must be empirically observed facts and that every theory is reliable to the extent that it is derived from these facts. The Florentine mathematician, astronomer and physicist Galileo Galilei (1564-1642) is also considered one of the founders of the experimental method.

Other important contributions to the experimental method originated in the 17th, 18th and 19th centuries, mainly with the works of the French mathematician and philosopher René Descartes (1596-1650), the English physicist and mathematician Isaac Newton (1642-1727) and the French scientist Antoine-Laurent de Lavoisier (1743-1794). The consolidation of the experimental method also had the relevant influence of the work of the renowned French physician and physiologist Claude Bernard (1813-1878), who consolidated his work in his famous book "Introduction à la médicine expérimentale".

The development of agricultural experimentation began at the end of the 18th century, at the time of agricultural development, stemming from the industrial revolution and the need to feed a rapidly growing urban population. Chemistry was beginning to be established in its modern form and its importance for understanding nutrition and plant growth was already recognized. Agriculture chairs were created in Oxford, England, in 1790, and in Edinburgh, Scotland, in 1797. Professor Humphrey Davy (1778-1829) of the Royal Institution of Great Britain taught an annual chemistry course in London for ten years, from 1803 to 1813, and published it in 1813 in the book “Elements of agricultural chemistry”, which had a major influence on the development of chemistry. In Germany, the renowned agricultural chemist Justus von Liebig (1803-1873) was appointed Professor of Chemistry at the University of Giessen in 1824. Thus, agriculture, with a firm base in chemistry, became accepted as an academic area.

The origin of modern genetic science applied to agriculture is credited to the experimental works of crosses with pea developed by the Austrian monk Johann Gregor Mendel (1822-1884). The now known Mendelian theory of inheritance was presented in his article "Experiments with plant hybrids", published in 1866. However, Mendel's work remained unknown until the early 20th century, when the extraordinary development of genetics began. At the same time, other scientists were conducting experiments and developing theories regarding plant crossing. Thus, for example, the

English naturalist Robert Charles Darwin (1809-1892) published, in 1876, the article "The effects of cross and self-fertilization in the vegetable kingdom", which published the results of experiments on self-fertilization and cross-fertilization in plants carried out in a small greenhouse.

In England, John Bennet Lawes (1814-1900) after his father's death in 1834, decided to discontinue his chemistry studies at Oxford and direct his properties in Rothamsted. He obtained conditions to work in the chemistry laboratories of the College of the University of London and then installed a laboratory in Rothamsted, where he began experimenting with superphosphate fertilizers derived from bones and mineral phosphates. In 1842, after prolonged experimentation of the effects of fertilizers on potted and field plants, he obtained a patent for his superphosphate production process from the treatment of rocks phosphate with sulfuric acid and started the first artificial fertilizer industry. In 1843, Lawes teamed up with English chemist Joseph Henry Gilbert (1817-1901) and founded, in Rothamsted, the world's first organized agricultural experimental station. Until 1900, for more than half a century, these two scientists devoted themselves to experimental research in plant and animal nutrition, making Rothamsted's work renowned worldwide. Due to the importance of this work, Lawes and Gilbert have been referred to as the fathers of the scientific method in agriculture.

Naturally, the design used in Lawes and Gilbert's experiments would not be accepted today, as they do not adopt the fundamental principles of the design of the experiment, that is, repetition, local control and randomization. This is despite the need for repetition to be recognized in the mid-19th century. Moreover, although the treatments of some of these experiments reveal the appearance of factorial structure, the levels and combinations of levels are very irregular. Even with their imperfections, these experiments were valuable as a starting point for the development of experimental research. Thus, for example, having been recognized that the results obtained in Rothamsted clay soils could not be repeatable in other soil types, from 1876 on, a parallel series of experiments was established in sandy soils of Woburn. This initiative seems to have been the first formal recognition of the importance of environmental variation between sites.

In addition to scientific importance, Rothamsted's experimental works are also relevant for their influence on the development of modern experimental methodology. However, these were not the only agricultural experimentation work of that period. Many agricultural research institutions were founded in Europe and the United States in the second half of the 19th century.

In 1889, John Lawes established the Lawes Agricultural Foundation to allow the continuation of Rothamsted experiments. After Joseph Henry Gilbert death in 1901, Alfred Daniel Hall (1864-1942) was appointed Director of the Rothamsted Experimental Station. Hall is remembered for his uniformity experiments with W.B. Mercer. Possibly, Hall and Mercer were the first to recognize that measures of error should be associated with data from agricultural experiments. Later, they established patterns for plot sizes of some types of experiments. At Cambridge, agronomist Thomas Barlow Wood (1869-1929) and astronomer Frederick John Marrian Stratton (1881-1960) showed a procedure for estimating errors in an animal nutrition experiment and reported one of the first uniformity experiments.

In 1912, Daniel Hall was succeeded by John Russell (1872-1965), a well-known agricultural chemist with a vocation for scientific administration. Russell began to worry about the huge data files generated by the 70-year results of Rothamsted's experiments. Knowing that the census institution had methods for extracting information from large masses of data, he decided to seek out a professional familiar with such methods who would be prepared to examine the Rothamsted data and obtain information that had not yet been obtained. For this task, he hired Ronald Aylmer Fisher (1890-1962), a young mathematician from Caius College, Cambridge.

Russell's expectations were more than exceeded. In the period from 1919 to 1933, in Rothamsted, Fisher developed theory and methods, as far as he was in need. He soon noted that better experiments could be planned than those of Lawes and Gilbert and their successors and began the development of the branch of statistics related to the planning and analysis of experiments. Fisher launched the modern foundations of experimental research, the basis of statistical inference and outlined many unique methods for the various problems encountered in Rothamsted and other research institutions. He introduced several techniques of data analysis, such as the analysis of variance, which became widely used in the statistical analysis of experiment data, and the orthogonal polynomials technique for the use of environmental characteristics. His first book, “Statistical methods for research workers” [4], an essentially practical text published in 1925, spread the new methods and made them available to researchers in agriculture and biology.

In the environment provided by Rothamsted, Fisher achieved the practical application of his theory of statistical inference and reached relevant conclusions for scientific research. One of these conclusions is that the amount of information generated by the inferences of a research cannot be greater than that contained in the data. Consequently, the data generation process and, particularly, the planning of the experiment, began to assume fundamental importance. Fisher soon understood that while the more elaborate statistical data analysis procedure could increase the precision of an experiment by a few percentage points, a more appropriate experimental plan, involving nearly the same effort, could increase precision by double or many times more, and could, in addition, provide information on important supplementary issues.

After 14 years of working on agricultural research, Fisher's ideas were already recognized and his modern methodology of planning and analyzing experiments was in full use.

Fisher emphasized the fundamental role of repetition as a basis for estimating error and consequently for the evaluation of evidence of the presence of effects. He launched the innovative notion of randomization and elaborated various forms of randomized design, particularly the randomized block design, which have become commonly used in field agricultural experiments. Fisher also stressed the importance of factorial experiments, arguing that nature prefers to answer questions proposed together rather than isolated questions. His most relevant contribution to experimental research was condensed into his book, "The design of experiments", published in 1935 [5].

The main characteristics of Fisher's approach are:

- Requirement that the experiment itself supply an estimate of the variability attributable to extraneous characteristics to which responses to treatments are subject,
- Use of repetition to provide this estimate of variability and randomization to allow for the validity of this estimate,
- Use of local control for the purpose of controlling and reducing extraneous variation,
- Principle that the statistical analysis of the results is determined by the way the experiment is conducted, and
- Concept of factorial design, that is, the research of the effects of two or more explanatory characteristics in a single experiment, instead of the dedication of a separate experiment for each of these characteristics.

Fisher's work on Rothamsted had a profound influence on the development of statistics and methodology of experimental research worldwide. Frank Yates (1902-1994) joined Fisher's team in 1931 and succeeded him in 1933. Yates continued to build on Fisher's ideas, notably in the development of factorial schemes with confounding and designs in incomplete blocks and lattices, which have become especially valuable in plant breeding experiments and in situations where block size is necessarily limited. Many other relevant contributions to agricultural experimentation originated from Fisher's school in Rothamsted, mainly from research by Frank Yates, John Wishart (1898-1956) and William Cochran (1909-1980). Environmental variation was recognized as an important problem in field agricultural experiments of wide spatial and temporal scope. The development of statistical methods for the planning and analysis of such experiments was initiated in the 1930s by Yates and Cochran [6] and Cochran [7].

The modern methodology of experimental research, developed from the foundations and ideas launched by Fisher for agricultural research, had many contributors in several countries and began to apply to other branches of science and technology, such as biology, medicine, engineering, industry and social sciences. The scientific and technological developments in the various areas required, in turn, new methodologies that also became, in general, applicable to other areas.

Because of the origin of experimental research in agriculture, much of the terminology still used today comprises terms specific to agricultural research. Thus, for example, the designations "treatment", "plot" and "block" have lost their connotations of agriculture and are widely used in experimental research in many areas of science.

3 The various concepts and classifications of experiments

The experiment is the research method of the widest application and tradition in scientific research. Consequently, there is a conceptual diversity and a methodological variation of the experiment, which gives rise to several classifications that are found in the literature, based on different criteria.

A first classification referring to the experiment considers the presence or absence of randomization. For this fundamental property, an experiment can classify as: random experiment or systematic experiment.

- In a random experiment, the assignment of an explanatory characteristic to the sample units is performed by randomization. In a systematic experiment, this assignment is done arbitrarily and subjectively, according to some systematic arrangement.

Systematic experiments have been reported in the literature in some situations; for example, in research on the spacing of plant species planted in pits with a "fan" design. In these experiments, the plants are arranged in concentric rays and the spacings grow systematically from the center to the periphery of the circle, with the purpose of reducing or eliminating surrounds, reducing the experimental area and allowing large number of spacings. In these circumstances, the spacing explanatory characteristic is not subject to randomization; therefore, it is not a treatment characteristic, which implies absence of the fundamental requirement for validity of inferences about its effects.

Thus, systematic experiments present inconveniences that do not occur with random experiments.

Some texts refer to experiments with a single treatment and classify experiments according to the number of treatments being one or more, into the following two categories: absolute experiment or comparative experiment:

- An experiment with a single treatment is called an absolute experiment; with more than one treatment is a comparative experiment.

An absolute experiment aims at inferences regarding effects on response variables of a treatment characteristic with a single treatment. For example, an experiment to describe the agronomic characteristics of a cultivar of a plant species and an experiment on the effect of a particular dietary supplementation on the milk protein and fat contents of Holstein cows. On the other hand, a comparative experiment comprises at least one treatment characteristic with at least two treatments. The main purpose of a comparative experiment is to derive inferences regarding comparisons of treatments, that is, inferences related to differences in treatment effects on response characteristics. Commonly, in these experiments are less relevant inferences about the individual effects of treatments. For example, in a comparison experiment of wheat cultivars, there is more interest in the differences between cultivar yields than in their individual yields.

In biology, particularly in agriculture, comparative experiments are much more common and important.

Comparative experiments are classified according to their objectives into two categories: scientific or basic experiment and technological experiment

- A scientific experiment or basic experiment has eminently cognitive purpose; it aims at a better understanding of reality, not necessarily for the purpose of its application. A technological experiment aims to generate useful knowledge with a view to its practical application.

These two categories of experiment are particularly distinguished by their differences in the relevance of the consideration of the target population and its representation by the sample. The specification of the target population is crucial in technological experiments. May not have as much relevance in basic experiments. Thus, for example, the clear definition of the target population is especially important in experiments on the efficacy of fungicides in the control of wheat giberela and the efficacy of anthelmintics in the control of sheep intestinal worms. It may be irrelevant in an experiment about symptoms of potassium deficiency in rice plants, which can be conducted on a highly homogeneous sample regarding the composition of extraneous characteristics, such as cultivation in a nutrient solution.

Technological experiments comprise three classes: preliminary experiment, critical experiment and demonstrative experiment.

- A preliminary experiment has exploratory purpose, often comprises a high number of treatments and aims to generate knowledge that allows the planning of critical, more specific and higher precision and validity experiments. A critical experiment aims to generate results (technologies) for recommendation of adoption by producers or other users of the research. A demonstrative experiment aims to verify and demonstrate the practical applicability of results of critical experiments in the real situation of the systems of the target population.

Preliminary technological experiments are illustrated by the following examples: a) A soil fertilization experiment for the cultivation of a plant species in a new region may include a relatively high number of treatment characteristics (macro and microelements) each with two levels, for the identification of the most impacting characteristics in production and their relevant interactions. This information is valuable for planning a critical experiment, with fewer treatment factors. b) In plant breeding programs, preliminary experiments with high numbers of lines generated by crosses and one or a few replications are common. Then, the best performing lines are selected for inclusion in critical experiments, in later phases.

The critical experiment should have sufficient precision and validity to provide reasonable safety to detect important differences between treatments. Usually, the considerations of the target population and its representation by the sample are not very relevant in preliminary experiments but have high importance in critical experiments. Demonstrative experiments are usually conducted in the circumstances of the units of the target population. Examples are the experiments carried out on the properties of farmers and breeders.

Critical technological experiments can classify according to sample extent into two classes: wide-ranging experiment and narrow-ranging experiment:

- A wide-ranging experiment (spatial or temporal) is an experiment in which the sample comprises two or more sections of space or time; a narrow-ranging experiment is an experiment in which the sample comprises a single section of space and time.

Preliminary technological experiments are commonly restricted in scope, since sample representativeness is not very important in the preliminary phase of an experimental research program. However, in critical and demonstrative technological experiments the representativeness of the sample is essential, which requires wide spatial and temporal scope.

This article focuses on randomized comparative experiments.

4 Characterization of the Experiment

- The experiment (comparative experiment) is the explanatory research method in which there is sample control: the levels of one or more explanatory characteristics are randomly associated with the sample units and extraneous characteristics are controlled to reduce the confounding of their effects with the effects of these explanatory characteristics and avoid the bias of the remaining confounding.

The examples below illustrate experimental research, considering concepts formulated in Silva [8].

- An experiment on the control of the incidence of giberela in wheat crops is carried out in four sites of a tricultural region in three years with two experimental factors: fungicide and cultivar, respectively with four and three levels. In each year, a terrain of each site is divided in four blocks of 12 plots for local control of characteristics of the soil. Then, the 12 combinations of the levels of fungicide and cultivar are assigned to the 12 plots of each of four blocks of each site and each year under the control of the researcher, which adopts random assignment process and control of experimental techniques to control extraneous characteristics to avoid bias of the effects of these characteristics with effects due to fungicide and cultivar. Therefore, fungicide and cultivar are treatment factors, each fungicide and cultivar and each of its combinations is a treatment, site and year are intrinsic factors, their levels in the experimental material are conditions as well as the fungicides and the cultivars and their combinations with sites and years. The effects of the extraneous characteristics constitute experimental error, which is controlled by local control, randomization and control of experimental techniques.
- An experiment on control of intestinal worms in lamb production units of a region is carried out in a production unit, with two experimental factors for response characteristics measured at slaughter: anthelmintic and sex, respectively with three and two levels in the sample; and an additional experimental factor: age for response characteristics measured in seven moments of the experimental period. The facility where the experiment is performed comprises 18 pairs of pens with common feeder and drinker, each pen with capacity for one animal, and 36 animals of the Ideal breed, 18 males and 18 females. The levels of anthelmintic are randomly assigned to the pairs of pens and the sexes male and female are assigned at random to the two pens of each of those pairs. Thus, if the control of experimental techniques guarantees absence of biased confounding of effects of the extraneous characteristics with anthelmintic effects, anthelmintic is a treatment factor and they levels are treatments. However, sex is an inherent characteristic of the animal; therefore, the effect of sex is confounded with extraneous characteristics of the animal. If the differences of the extraneous characteristics between the two sexes is irrelevant, sex can be considered a treatment factor. This is the case if the animals of the two sexes come from the same herd and proper control of extraneous characteristics is exerted. Otherwise, sex should be considered an intrinsic factor. The experimental factor age is an intrinsic factor.

The control of the assignment of the levels of treatment factors to the units of the sample should be exercised by some process that guarantees the absence of biased confounding of the effects due to these characteristics with effects of extraneous characteristics. This guarantee is provided by randomization. Randomization has relevant consequences for inferences regarding the causal effects of treatment factors on response characteristics. Randomization does not apply to intrinsic factors.

The impossibility of absolute control of the explanatory characteristics and extraneous characteristics in any scientific research implies that an experiment can never prove the hypothesis that originates it. However, a valid experiment that provides results that contradict the hypothesis is sufficient to reject it.

The control exercised over the presence of explanatory characteristics is an exclusive property of the experiment that distinguishes it favorably from other explanatory research methods. However, the representation of the target population by the sample is a crucial issue in the experiment. Firstly, because, commonly, the sample is not obtained by random selection process of units of the target population. This selection process is usually unfeasible. Generally, the sample is constituted by units built especially for the research. For example, in a field agricultural experiment with beans, the sample unit is a cultivation in a small plot, while the unit of the target population is a crop that can cover several hectares; in a nutrition experiment of swine raised in confinement, the sample unit can be a piglet in a pen, while in the target population is a set of piglets in a larger facility.

In these circumstances, the validity of inferences derived from the experiment necessarily depends on subjective evaluation. The inferences are valid (i.e., unbiased) for the population of units that can be considered represented by the sample, that is, for the sampled population. The adequacy of the extension of the inferences to the target population depends on the proximity between the sampled population and the target population, that is, the sampling error. It should be evaluated subjectively based on information that can be obtained about the nature of the differences between the sampled population and the target population.

These considerations are important in technological experiment, which aims to derive inferences for application in real situations. They may not have as much relevance in basic experiment, with only cognitive purpose.

5 Experiment Process

The experiment fully reveals the systematic property of the scientific method. Its process comprises an ordered sequence of steps from its origin, with the formulation of a scientific problem, to the achievement of its objectives, with the solution of this problem. From the initial question to its final answer, an experiment proceeds through eight main stages of operation, listed below:

- First stage: Identification and establishment of the scientific problem or research problem.
- Second stage: Formulation of the scientific hypothesis or research hypothesis.
- Third stage: Review of the available information.
- Fourth stage: Construction of the research plan.
- Fifth stage: Data collection.
- Sixth stage: Data analysis and interpretation.
- Seventh stage: Derivation of conclusions, which may lead to confirmation or rejection of the original hypothesis, and confirmation or questioning of results of other studies.
- Eighth stage: Presentation of the results through a report, and dissemination of these results.

Here, only the considerations most relevant to experimental research will be made.

5.1 Establishment of the scientific problem

The problems are the springs that drive scientific activity. Scientific research consists essentially in finding and formulating problems and trying to solve them.

The identification of research problems is carried out in the initial phase of synthesis and in the beginning of the analysis phase of a cycle of the scientific method applied to improve the performance of the units of a target population.

In practice, it is often not simple to identify the existence of a scientific problem. Even the narrowest parts of the real world are too complex to be fully and accurately understood. Consequently, it is necessary to ignore most of the characteristics of the units under study and abstract an idealized version of these units. This idealization, if successful, provides a useful approximation of the actual units. Usually, it is convenient to decompose this idealized version into several parts for separate consideration, that is, analyze the units (systems) and the corresponding overall problem.

The possibility of such a procedure is based on the existence of components that are approximately independent or interact relatively simply.

This analysis of the global problem leads to the formulation of a set of specific problems, each of which can be tentatively solved by scientific research methods and available resources. Each of these problems constitutes a particular scientific problem:

- A scientific problem or research problem related to the units of a target population is a specific problem, question or query related to relationships of connection of characteristics of these units that is answerable through the scientific method, with the methods, techniques and resources available.

5.2 Formulation of the hypothesis

After the formulation of the problem, the next step is the inquiry regarding nature and connections of characteristics that lead to the idealization of one or more paths to solve the problem. Each of these idealized paths constitutes a research hypothesis:

- A scientific hypothesis or research hypothesis is a proposition of solution to a research problem that is derived from a theory by deductive inference, from reasoning based on observation of events or from the literature, and which allows for a test of empirical confirmation.

The function of the scientific hypothesis is to extend scientific knowledge beyond the present frontiers of theoretical knowledge. Thus, the scientific hypothesis is more than a connection between speculation and verification; it is the essential factor of the growth of scientific knowledge.

In an experiment, a hypothesis refers to a causal relationship of characteristics of the units of the target population. It seeks to find out whether the alteration of one characteristic or set of characteristics implies a change of another characteristic or set of characteristics.

For the same problem one or more hypotheses can be formulated. These hypotheses may differ in degree of subtlety, due to the level of complexity of the problem. A simple hypothesis can be a mere generalization of a particular empirical observation. More complex hypotheses can postulate connections between events, or elaborate chains of casual relationships.

Very often more than one hypothesis can be formulated as a likely solution to the research problem. In these circumstances, The simplest and most fruitful hypothesis to achieve the objectives of the experiment should be chosen.

The establishment of the problem and the formulation of the hypothesis define the objectives of the experiment. These decisions require a clear and unequivocal characterization of the purpose of the experiment, the target population, its units and important characteristics of these units. In particular, the following questions should be clearly answered:

- Purpose of the experiment: Does the experiment aim to generate knowledge for the development of theory, or to provide technology for immediate use? Is the experiment a preliminary step in a research program to determine its future course, or a step that aims at making decisions for practical application?
- Target population: To which spatial and temporal scope should the inferences of the experiment be applied? That is, what is the geographical amplitude and the time interval of the target population.
- Units of the target population: What is the level of units (systems) that should be considered? For example: i) are the units the whole of animals in a facility (paddock, box or cage) or an individual animal or one of their parts? Ii) are the units the whole of the plants in a plot (field plot, plant bed or pot) or an individual plant or a part of it?
- Characteristics: Are the characteristics of these units the same as those of the current units or supposedly evolved characteristics? For example: i) are cultivars compared to crops with more advanced cultivation techniques, such as higher fertilization levels and more intensive control of diseases and pests? ii) are antibiotics under research intended for selected animals with higher productivity levels?
- Levels of characteristics: Are the levels of these characteristics the same as those of the units of the target population or a fraction of them? For example, are feed compositions intended only for animals of a specific sex and age range?

5.3 Review of the available information

After careful and logical evaluation of the formulations of the scientific problem and the scientific hypothesis, the next step is the search for all information that can provide useful support for the new research, mainly to evaluate the importance of the proposed problem and to determine whether the problem and the corresponding hypothesis are plausible and will lead to the addition of new knowledge. This search comprises, mainly, the consultation of the available literature regarding the research area, the consultation with experienced researchers and the collection and identification of available material and data that may be useful for consideration in the research to be conducted. It should also include the collection of information regarding similar research already performed and the techniques and procedures that may be useful for adoption in the new research.

The review of the available information, particularly the literature examination, has the following relevant objectives and functions:

- Evaluate the importance of the proposed problem and verify whether the problem and the corresponding hypothesis were the subject of previous research, whether they are plausible and will lead to the addition of new knowledge,
- Seek the appropriate approach for the research, through the collection of information related to similar research already performed,
- Identify specific methodological problems that require a solution before conducting the research, or that should be considered in the research plan,
- To gather suggestions on techniques, instruments and other material resources that may be useful for the purposes of the research,
- Identify possible difficulties and dangers that may arise in the conduct of the research,
- Contribute to the discernment of possible solutions to the problem,
- Provide the available data, an indispensable condition for the efficiency of the research.

5.4 Planning of the experiment

The scientific approach requires the planning of the experiment, that is, the early establishment, in written form, of the complete set of decisions and actions that must be taken and carried out for the execution of the experiment. The plan of the experiment should be consistent with the research objectives determined by the problem and the hypothesis and be formulated in order to ensure the derivation of the inferences that the experiment aims to establish for the achievement of these objectives.

Very often, the importance of this stage is underestimated, that is, the time and attention needed to elaborate the plan of the experiment are not dedicated. This failure is the source of many experiments that do not produce useful results or do not derive the information that could potentially produce with the same resources.

This step comprises, fundamentally, the planning of the sample, particularly of the structure of the experiment, the planning of the actions and procedures for the execution of the experiment, and the definitions of the resulting statistical model and the statistical analysis procedures for the derivation of inferences.

5.4.1 Sample planning

The sample shall be defined to ensure the appropriate precision and validity.

The precision of the experiment should be sufficient to provide high probability of detecting differences in important responses existing in the target population that are attributable to treatment factors. When planning the experiment, the researcher should consider that the precision can be increased by using a more homogeneous sample and increasing sample size. As previously pointed out, in technological experiments the first resource has limitations. In these circumstances, determining and adopting the appropriate sample size is of high relevance. The appropriate sample size to achieve the desired precision depends on the magnitude of the important response differences, the variability of the extraneous characteristics of the experimental material, and the magnitude of the risks of incorrect decision that can be tolerated in the inferences to be derived. Very often, the sample size is not determined and, when it is, it is often too high to be adopted. In general, the researcher has to establish a compromise between statistical efficiency and economic and practical efficiencies and adopt other means to increase the precision required for the experiment.

For the guarantee of high validity, the sample shall consist of units representing the range of variability of the units of the target population. If this population consists of units dispersed in space and time, then the sample must consist of units arranged in different positions of space and time.

The sample planning comprises the choices of the response characteristics, the experimental factors and the extraneous characteristics to be controlled, the variables for expression of the characteristics that should be measured and the corresponding measurement procedures, and the definition of the interrelationship between these three classes of sample characteristics. These choices are made by the planning of the response, planning of the experimental conditions, planning of the experimental control and planning of the experiment structure or experimental design. The structure of the experiment forms the basis for the derivation of inferences for the achievement of the objectives of the experiment and must be determined by these objectives.

5.4.2 Planning of actions and procedures for the execution of the experiment

The techniques and procedures that should be adopted in the conduct of the experiment should be planned so that the necessary conditions and resources are available when required, and the decisions and actions for their execution are established at the appropriate times. This planning is necessary to minimize the occurrence of unforeseen situations that may cause damage to the experiment, especially those that may lead to extraneous sources of disturbing variation.

5.4.3 Definition of the statistical model and data analysis procedures

Statistical inference procedures require the establishment of a statistical model, that is, a mathematical model that represents the relationship between the response variables and the explanatory variables and takes into account the presence of the extraneous characteristics of the sample.

At the conclusion of the plan of the experiment, a statistical model that represents the structure of the experiment, that is, the relationship between the explanatory characteristics and the extraneous characteristics and their effects on the response characteristics should be formulated. The statistical model constitutes the basis for the definition of the consequent statistical procedures appropriate for the analysis of the data with a view to the derivation of the inferences object of the experiment. The definition of these procedures of statistical analysis should then be outlined. The resources needed to perform these analyses should be provided to ensure their availability at the data analysis stage.

5.5 Conducting the experiment

The researcher should follow and conduct the experiment carefully to ensure compliance with the pre-established plan and record of extraneous occurrences that may have relevant influences. Attention should be paid to the experimental design and to the execution of the experimental techniques for experimental control to avoid the appearance of disturbing extraneous characteristics, which affect the characteristics in a systematic way, causing biased confounding with effects of treatment characteristics.

This is the stage of data collection, that is, the measurement and recording of the observed values of the response variables, covariates and other important variables, as established in the plan of the experiment. Relevant unforeseen occurrences must also be recorded for proper consideration in the stages of analyzing the results and drawing up conclusions.

It is important that data recording is followed by careful review and data criticism that ensures the detection of possible errors, especially gross errors.

5.6 Analysis of results

Statistical analysis methods and procedures proper to the objectives of the experiment, consistent and coherent with the experimental design adopted and with the corresponding statistical model established in the experiment plan, must be used.

In much research, a preliminary stage of data inspection can be useful, through description and summary of the data, and the use of techniques to verify the assumptions established with the statistical model. This procedure may provide the most evident indications revealed by the results of the experiment, which may be important for guidance regarding the statistical analysis procedures to be adopted.

The availability of computational resources (equipment and programs and systems for statistical analysis) make it possible to use appropriate methodologies for data analysis.

An important part of the data analysis process is the verification of the adequacy of the statistical model, that is, the critical examination of the statistical model adopted, particularly its assumptions. Computing resources and the use of graphic techniques are particularly useful for this purpose.

It should be warned that computing resource facilities can induce misuse. Misuse may arise, for example, from the use of data analysis procedures available in statistical analysis packages without verifying their suitability, especially regarding the required assumptions.

5.7 Interpretation of the results, preparation of conclusions and their dissemination

After data analysis, the experimenter should draw inferences or conclusions from the results. It is appropriate to highlight the limitations of the validity of statistical inferences. Thus, for example, the use of statistical methods does not prove that one or more explanatory characteristics have effects in an experiment. It only provides guidance regarding the precision and validity of such inferences. In fact, the use of appropriate statistical methods does not allow definitive proof of arguments based on the results of an experiment but allows the evaluation of the probable error of a conclusion, or the assignment of a confidence level to a proposition derived from the results of the experiment.

Statistical inferences must be interpreted physically, and practical meanings should be evaluated and considered along with statistical significance. In some situations, statistical significance may differ from the practical meaning. For example, an experiment may show small differences between treatments as significant when they have no practical significance, given the resources and costs required. On the other hand, non-significant differences between treatments may have practical significance when resources and costs are considered.

The application of the results of an experiment conducted to research a problem usually requires a joint evaluation with the results of other actions of the research program and with existing information. That is, an approximation to the real system should be constructed by a synthesis of the knowledge generated by the experiment with the previous body of knowledge. Two requirements are indispensable for the success of this synthesis: the new knowledge must be correct and the interactions of the problem solved with the other problems of the systems must be considered sufficiently approximate to reality.

The results of the experiment should derive recommendations. These recommendations may include a further set of experiments, since experimental research is an iterative and sequential process in which an experiment answers some questions and proposes other questions.

This last stage of the experiment is only concluded with the dissemination of the results, by appropriate means, for its effective incorporation into the body of scientific knowledge and its use.

Thus, the experiment process comprises an ordered sequence of steps that originates an implied chain such that the decision made at each step has consequences for the following steps (Figure 1). Any incorrect decision or action taken in one step implies incorrectness for the following steps. This means that achieving the objectives of an experiment requires correct definitions and decisions in all its stages.

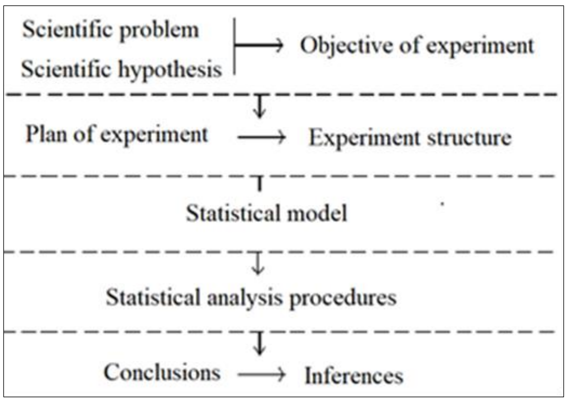

Figure1 Implied chain of the steps of an experiment

Planning for achieving the aims of the experiment can lead to one or more alternative designs. Usually, one of these designs is the most suitable for efficiency and practicality. This is the design that should be chosen. Once the design of the experiment is defined the appropriate statistical analysis procedure should be decided. It is relatively easy to determine the statistical analysis procedure for a well-designed experiment. The reason is that the method for statistical analysis of the data is an immediate consequence of the objectives and design of the experiment. Thus, if the objectives of the experiment were well established and the plan well elaborated, the choice of the method of analysis is usually not difficult and is essentially predetermined. For this reason, it must be specified in the experiment protocol (section 7). Although it is often possible to consider more than one version of the analysis procedure, these versions, if equally appropriate, usually differ irrelevantly and will lead essentially to the same conclusions. The occurrence of discrepancies should be taken as a warning that some assumption or some essential aspect of the experiment should be investigated. On the other hand, an experiment with poorly conceived objectives or with planning flaws presents very often insuperable difficulties for the establishment of the statistical analysis procedure.

5.8 Reference List for Running an Experiment

In each of the stages of the experiment, the researcher must make decisions and establish actions, each of which can be of vital importance to maximize the chance that the experiment will be conducted without unforeseen events until the achievement of its objectives. A detailed list of the actions and care that must be taken in the execution of an experiment, such as the one that follows, is very useful as a reference and roadmap for planning and running the experiment.

- Get a clear view of the problem.
  - Identify the target population and its units.
  - Identify the specific problem that is an obstacle to the performance of the target actions.
  - Outline the specific problem within the current limitations.
  - Determine the relationships of the problem with the other important problems of these units.
  - Arbitrate conjectures of possible solutions to the problem.
  - Choose the most fertile conjecture as the hypothesis for the experiment, considering the expected impact for the improvement of the performance of the units and the resources available for the research.
  - Determine the relationship of the experiment with the overall research program for the target population.
- Collect available background information.
  - Investigate all available sources of information.
  - Organize and tabulate data relevant to the experiment plan.
- Plan the experiment.
  - Hold a meeting of all researchers from the disciplines involved in the research area.
    - Determine the propositions to be verified.
    - Establish the list of response characteristics of interest, in the order of their importance, according to the objectives of the experiment.
    - Specify the variables and their measurement scales for the expression of these characteristics, and the corresponding procedures and instruments to be used for measurement.
    - Establish the magnitude of important differences between values of the most important response variables.
    - Choose the experimental factors: a) the experimental factors directly related to the scientific hypothesis, and b) other experimental factors relevant to the achievement of these objectives, especially those that may affect the effects of those factors or allow the proper representation of the target population.
    - Determine the practical amplitude of the levels of each of these experimental factors in the target population and the specific levels to be adopted in the sample.
    - Consider possible interrelationships (i.e., "interactions") of experimental factors.
    - Choose the combinations of the levels of these experimental factors for the sample.
    - Determine the scope of the sample, considering the adequate representation of the target population. Establish the execution of the experiment in various locations, years, laboratories, etc., as appropriate to achieve this representation.
    - Clearly establish the characterization of the sample and the sampled population, that is, of the population of units that can be considered represented by the sample, and its relationship with the target population.
    - Consider the possible consequences of variability of the sample's extraneous characteristics for the precision of the inferences to be derived from the experiment.
    - List the extraneous characteristics that may manifest in the sample, through its large aggregates; in each of these aggregates identify the characteristics that may constitute relevant sources of variation of the response variables.
    - In each of these aggregates identify the potentially relevant extraneous characteristics that can be controlled by experimental techniques without prejudice to the representation of the target population. Plan to control these characteristics.

- Among the other characteristics of each of these aggregates, identify those that can be controlled by local control and statistical control. Plan to control these characteristics.
- Among the remaining characteristics of each of the extraneous characteristic aggregates identify those that may be controlled by randomization. Establish the randomization procedure for these characteristics, particularly the procedure for the random assignment of treatments to sample units.
- Determine the number of repetitions, considering the uncontrolled extraneous variability and the important differences that the experiment aims to detect.
- Establish the structure (design) of the experiment and an outline of the resulting statistical model.
- Determine the limitations of material, instruments, human resources, costs, budget, and time.
- Consider the aspects of human relationships of the program.
    - Complete the planning of the experiment in preliminary form and prepare its execution.
        - Prepare a systematic document of the experiment plan, including details of the procedures to follow.
        - Provide the execution of the experiment in stages and the adaptation of the plan of its execution, if necessary.
        - Establish the experimental techniques to be adopted during the execution of the experiment.
        - Develop and adapt the necessary methods, materials, equipment and instruments.
        - Choose the methods and statistical analysis procedures consistent with the statistical model.
        - Plan for the orderly collection of the data, in particular a spreadsheet for recording the data.
        - Prepare the execution of the experiment; prepare the sample, establish its units and the groupings constituted by the established local control; perform the randomization and establish the arrangement of treatments in the experimental units by means of a sketch or list.
    - Review the plan with the multidisciplinary team.
        - Discuss again all the details of the experiment plan.
        - Adjust the plan appropriately, if necessary.
        - Establish the steps to be followed in a clear and explicit manner, defining the participation of each team member in the execution of the experiment.
- Run the experiment.
    - Apply the treatments to the sample units by random process, according to the pre-established plan.
    - Apply the methods and techniques defined in the experiment plan.
    - Follow and check the details; change methods and techniques if necessary.
    - Write down any modification of the plan.
    - Take precautions for data collection; check the reliability of the measurement instruments; check the correction of the recorded data; avoid data transcription.
    - Write down the progress of the execution of the experiment; record the relevant events and occurrences.
    - Check the occurrence of relevant events that may imply confounding effects of extraneous characteristics with experimental factors effects; determine the convenience and appropriateness of disregarding results in units affected by such occurrences.
- Analyze the data.
    - Reduce the recorded data to the numerical form if necessary.
    - Edit the data, preferably in magnetic medium; determine the data of the derived response variables, whose values are to be determined through expressions from the data of the primary response variables, which are recorded directly.
    - Perform an exploratory and descriptive analysis of the data, and check for possible presence of discrepant or aberrant values.
    - Apply proper statistical techniques for data analysis, consistent with the objectives of the experiment and the statistical model established in the experiment plan.
    - Write a summary of the results of the data analysis, build illustrative tables and graphs.
- Interpret the results.
    - Consider all observed data, including those not submitted to statistical analysis.
    - Limit conclusions to narrow deductions from the evidence at hand.
    - Check the conclusions regarding statistical significance and its technical and practical meanings.
    - Contrast the results obtained with the results of other research and the existing scientific knowledge; characterize the insertion of the solution of the initial problem provided by the experiment in the units of the target population.
    - Highlight the implications of the findings for the application and for further research.
    - Consider any limitations imposed by the methods used.
- Prepare the report.
    - Describe the work clearly, characterizing the antecedents, the relevance of the problem and the meaning of the results.

- Present the results in a manner appropriate for their better understanding and use, using appropriate tables and charts.
- Provide enough information to allow readers to verify the results and draw their own conclusions.
- Limit the conclusions to the objective summary of the evidence provided by the experiment, so that the work is recommended for prompt consideration and decisive action.

It is not necessary for the steps suggested in this list to be followed and executed in this order. This list is only a guide and should be adapted for each situation. Scientific research, particularly experimental research, is not a mechanical and routine activity. During the planning of an experiment many questions are raised, discussed by researchers and answered, giving rise to new ideas and leading from one step to another in a seemingly casual way. Moreover, it is usual that with the addition of new information and continuous evaluations the same step is considered more than once.

Planning an experiment is a time-consuming and tiring process. Therefore, the use of a reference list, such as the one above, can be a supplement to common sense, very useful to ensure that important considerations and particularities are not forgotten.

6 Experiment Protocol

The experiment protocol is the written documentation of the experiment. Its elaboration must begin in the experimental planning phase and constitutes an essential element that must precede the execution of the experiment. The completion of the experiment plan documentation is the last opportunity for a deep reflection before starting the actions for its execution. Then, all the researcher's energy must be focused on executing the established plan and providing solutions for the problems that may arise. This reflection should include a careful review of the reference list in section 6.

The documentation of the experiment is stablished in its protocol. In its first part, the experiment protocol should comprise all relevant information regarding the research plan. Its content depends on the research area. In general, it should include the following topics:

- Reference - Indication of the program, project and subproject, if applicable, to which the experiment is linked.
- Title of the experiment - Designation that expresses the aim of the experiment in a summarized and clear way.
- Scientific problem - Formulation of the research problem, characterizing particularly the target population and its units (section 5.1).
- Scientific hypothesis - Formulation of the research hypothesis (section 5.2).
- The formulations of the research problem and the research hypothesis should fully characterize the objectives of the experiment.
- Material and methods - Description of the experimental material and the procedures and actions to be adopted and implemented for the execution of the experiment. That description shall be sufficiently detailed to allow the identification of the response characteristics, experimental factors and relevant extraneous characteristics. It depends on the research area, but in general it should comprise the following items:
  - Experiment execution period - Start and end dates of experiment run.
  - Locations and years of execution - In the case of a wide-ranging experiment, listing of the years and sites planned for conducting the experiment and definition of the facilities to be used in each of the sites. In these experiments, the collaborating institutions and researchers should be identified. In the case of field agricultural experiment, latitude, altitude, climatic conditions, topography, soil type, etc. should be identified for each site.
  - Response characteristics, variables chosen to express them and corresponding measurement scales and measurement processes - Individual identification and listing of all response variables. In the case of characteristics expressed by interval or rational variables, the scales and units of measurement, the accuracy of measurements (numbers of decimal digits to be recorded) and the instruments to be used must be clearly defined. In the case of nominal and ordinal variables, the scales and levels (categories) and criteria to be adopted and the instruments to be used must be clearly established.
  - Experimental factors and their levels in the target population and the corresponding levels and combinations of levels to be considered in the sample (conditions), particularly of treatment factors (treatments). In the case of a quantitative factors, the numerical values of the levels shall be clearly specified with the adoption of some conventional unit of measure, such as kilogram, liter and meter. In situations of more than one treatment factor and additional treatments, combinations of levels and additional treatments should be identified. Intrinsic factors and their levels should also be defined clearly.

- Other materials - Listing and characterization of all specific materials that should be used and that do not constitute experimental factors, such as fertilizers, insecticides, fungicides, herbicides, vaccines and antibiotics.
- Characterization of the units - Complete characterization of the sample unit, by specifying its dimensions, the number of animals or plants that constitute it, its sketch, etc. In the case of field agricultural experiment, the dimensions of each plot, the form of planting (pit, toss, line, etc. must be established), the planting density, the number of plants, the surrounds, the distances between plots and the arrangement of the plots in the field. In animal experiments, units (which can be individual animals, groups of animals in a pen, box or cage) must also be established and clearly identified. In any experiment, the units (experimental units) to which the levels of each of the treatment factors should be applied must be clearly identified. In the case of more than one formation of experimental units, this fact must be clearly established.
- Experimental techniques to be used and timing of its execution - Listing and description of the procedures and actions to be performed during the conduct of the experiment, from its installation to the completion of the measurement process and corresponding data recording. For example, a) in plant experiments: cultural techniques, such as soil preparation, planting, weeding, applications of insecticides, fungicides and herbicides, collection, measurement and recording of values of important variables, particularly those that express response to the effects of treatments; in animal experiments: management techniques, such as applications of antibiotics, vermifuges, vaccines and shearing; and procedures for collecting and recording data. This listing and description should be sufficiently detailed for perfect understanding and should specify the schedule of the execution of the experimental techniques.
- Experimental design - Identification of the experimental design adopted (for each site and year, in the case of an experiment of wide spatial and temporal scope). Very often, the design of the experiment is identified by the usual description in the literature. However, it is more appropriate and convenient, particularly for complex experiments, to characterize the experiment design by a detailed description of the experimental procedure. Groupings and blocks formed by local control must be clearly identified.
- Covariates – Listing and description of covariates representing relevant extraneous characteristics of the sample that should be considered in the statistical model and in the statistical analysis of the results of the experiment for statistical control, and of explanatory covariates. The characterization of these variables should comply with the same care and procedures of measurement described for the response variables.
- Sample sketch – Outline of the spatial layout of the units and the corresponding levels of the experimental factors associated with them. In wide-ranging experiments, this sketch should be performed separately for each section of space and time. This sketch is very useful for guiding the installation and monitoring of the experiment and data collection. Naturally, a sketch only makes sense in situations where units are spatially arranged in fixed positions while conducting the experiment. If the units are not arranged in fixed positions, as with some animal experiments, an alternative to sketching is a list with the identification of the units and levels of the experimental factors associated with them.
- Field booklet -- Spreadsheet suitable for recording data and information about relevant occurrences while conducting the experiment. This spreadsheet should be attached to the experiment protocol.
- Statistical model and scheme of statistical data analysis procedures - Specification of the algebraic equation that relates each important response variable with variables representing effects attributable to experimental factors, groupings of experimental material units, constituted by local control and from other sources, and covariates. The statistical model must adequately express the structure of the experiment. It will serve as the basis for the definition of procedures for statistical analysis of experiment results. These procedures should be described in summary form.

- Means and processes to be adopted for the dissemination of results.
- Budget List of necessary financial resources foreseen for the execution of the experiment, such as acquisition of material, equipment, labor, travel, maintenance, etc.
- Collaborators - Identification of people and institutions participating in the experiment, contributing with financial resources, facilities, material, equipment and other facilities.
- Responsible - Name of the professional responsible for the experiment.

The protocol shall be further continued with the documentation for the relevant occurrences during the execution of the experiment. The documentation of the experiment should be extended and completed, in due times, with the data generated, the results of the analysis of the data and the reports and publications prepared for dissemination of the results.

The written and careful documentation of all the details of the experiment is an elementary rule, but of great relevance. The need to refer to the previous information often arises during the conduct of the experimental work. This documentation is especially important in experiments of wide spatial and temporal scope. It provides data preservation and security of the future usefulness of the research carried out. It is also relevant to allow the use of data in exploratory research for purposes different from those that originated the experiment, for the detection of research problems, the indication of hypotheses and the evaluation of research progress.

7 Statistics in Experimental Research

The experimental method had a strong expression in physics and chemistry (section 2), justified by a very special and very powerful article of faith. The argument consisted of the following: if each of the conditions for the occurrence of a phenomenon is controlled, except by a particular deliberate stimulus, the exact relationship between the response to the stimulus and the stimulus can be observed. Naturally, this process involved the construction of artificial situations. The effectiveness of this plan is evidenced by the enormous development of physics and chemistry., especially in the 17th and 18th centuries. However, it presupposes the construction of essentially identical units with respect to the response. This plan for the construction of scientific knowledge fails when it is impossible to build identical units and when the interest lies in obtaining information about populations of units that are available in nature and that manifest variability, often of a huge amplitude.

A way to solve this problem was developed by Fisher [5] based on the idea of randomization and the use of the statistical method. Fisher's approach is based on the construction of an experimental design that incorporates the statistical principles necessary for the derivation of valid inferences from the data generated by the experiment (section 2). Thus, if data are collected based on an appropriate experimental design, these inferences can be derived by statistical analysis. For example, in a soil fertilization experiment for wheat cultivation, estimates of the unknown means of yields of different fertilizers and the estimate of experimental error can be determined. Then, the decision on the significance of the observed mean differences considering the uncontrolled extraneous variation present in the experimental material can be provided by a statistical procedure. The statistical approach provides the rigorous expression of the degree of uncertainty of these inductive inferences that generalize the experiment results to the sampled population.

Whatever evidence provided by the experiment, i.e., in favor of or contrary to the presence of differences in effects of the treatments, it may be incorrect. The statistical method allows establishing the degree of uncertainty, in terms of probability, of either of the two decisions that are made as a result of the evidence indicated by the experiment, that is, that the treatments differ or that the treatments do not differ.

Assuming that the experiment was conducted correctly, its results can be questioned for two reasons. The first criticism is that the design defined for the experiment may be flawed. The second criticism is that the analysis and statistical interpretation of the data may be incorrect. The developments of statistical methodology and computing resources have made available a wide range of statistical methods and procedures for data analysis that allows the researcher to use the appropriate methods and analysis procedures for virtually whatever design defined for his experiment. Of course, the proper use of these facilities depends on the knowledge of the statistical methodology, particularly the assumptions required for the validity of its application. It is up to the researcher and statistician to evaluate the suitability of statistical methods and procedures for each situation. These two criticisms are really two aspects of the same whole, because if the design is flawed, statistical analysis and interpretation will also be flawed, unless design failures are discovered and can be considered. As pointed out by Fisher [5], the design of the experiment and the corresponding method of analysis of the results are inseparably related. This means that statistical analysis procedures should be defined in line with the design adopted for the experiment.

Applied Statistics comprises a set of methods developed specifically for experimental research:

- The statistical methods applied to experimental research are usually called Experimental Statistics.

8 Conclusion

The experiment is the method of scientific research par excellence. The experiment makes it possible to fulfill the requirements of the scientific method for the acquisition of reliable knowledge. These requirements include a process that comprises a sequence of logically linked steps and the observance of properties that ensure the generation of precise and valid inferences. These properties require sample control that guarantees unbiased confounding of treatment factor effects with experimental error. The researcher must be aware of these necessary conditions for his experiment to be successful.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declare that no conflict of interest is exist.

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