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Geomatic Techniques to Support Phytosanitary Products Tests whithin the EPPO Standard Framework

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Chapter 1

Introduction

1.1 Phytosanitary Products

Phytosanitary products, commonly used as a synonym for ”Plant Protection Products” (PPPs), are a specific category of pesticides designed primarily to maintain crop health and prevent destruction by diseases and infestations. While the term ”pesticides” is broader and also includes biocidal products used to control harmful organisms and disease carriers not related to plant protection, phytosanitary products are specifically used to control harmful organisms affecting cultivated plants (such as insects, mites, fungi, bacteria, rodents, etc.), eliminate weeds, and regulate plant physiological processes. Fertilizers, which serve for plant nutrition and soil fertility improvement, are excluded from phytosanitary products.

Phytosanitary products contain at least one active substance, which can be either chemical compounds or microorganisms, including viruses, that enable the product to perform its intended function. These active substances undergo rigorous risk assessment processes, with EFSA (European Food Safety Authority) playing a central role in conducting peer reviews at the EU level to determine if these products, when used correctly, might produce harmful effects on human or animal health, either directly or indirectly through drinking water, food, or feed.

The main categories of phytosanitary products can be distinguished based on the type of organism they target or the function they perform, including:

- Fungicides
- Insecticides
- Acaricides
- Rodenticides
- Slimicides
- Nematicides
- Herbicides
- Plant growth regulators

The parameters identified through the risk assessment are compared with the values established by directive 97/57/EC [8], which indicates the acceptability limits for decision-making on the inclusion of active substances in the EU list (Annex I of directive 91/414/EEC [3]).

The Introduction of a product in the EU market is not only subject to audits on active substances and their safety for humans and environment but also to the evaluation of the product's efficacy and safety for the crop. World Trade Organization Sanitary and Phytosanitary Measures Agreement [14] recognizes the International Plant Protection Convention (IPPC) as the only international institution in charge of emitting standards for plant health [12]. IPPC is organized in regions. European Union (EU) countries refer to the European and Mediterranean Plant Protection Organization (EPPO). EPPO Standards are divided into Standards on Phytosanitary Measures and Standards on PPPs. PPPs standards describe the efficacy evaluation of PPPs (PP 1) and good plant protection practices. EU GEP units provide Biological Assessment Dossier (BAD) efficacy trials. GEP units are expected to follow EPPO PP 1 to assess PPPs selectivity detecting phytotoxicity effects, and efficacy in the complaint of Regulation (EC) No 1107/2009 of the European Parliament and Council [9].

1.2 EPPO Standards

Generics on efficacy assessments are reported in PP 1/181(5) [7], which describes herbicide, fungicide, bactericide, and insecticide efficacy on the target evaluation. PP 1/135(4) [5] describes the selectivity assessment procedures, in other words: the standard phytotoxicity assessments of PPPs. The PP 1/152 [4] standard describes the general principles for the efficacy and selectivity evaluation of PPPs, in describing the standard experimental design. Aside from the objectives of the study and the description of thesis (treatments), the PP 1/152 outlined that a comprehensive experimental design should include a description of:

- **Type of Design**
- **Sampling Method and Measures Units**
- **Statistical Analysis Plan**

1.2.1 Experimental Design

EPPO ”envisage trials in which the experimental treatments are the ‘test product(s), reference product(s) and untreated control, arranged in a suitable statistical design’” [4]. The experimental design should be randomized, with replications and blocks, and should include a sufficient number of plots to ensure the statistical power of the analysis. The number of replications and blocks should be determined based on the expected variability of the data and the desired level of statistical significance in respect control and reference thesis. The randomization of thesis within blocks should be carried out using a suitable randomization procedure to ensure that the treatments are assigned to plots in a completely random manner. The key randomization used in phytosanitary product evaluations include:

- **Completely Randomized Design (CRD)**: Treatments randomly assigned to experimental units; statistically powerful but only suitable for homogeneous trial areas where environmental variation is minimal.

- **Randomized Complete Block Design (RCBD)**: Groups plots into homogeneous blocks with each treatment appearing once per block; controls for environmental heterogeneity across the experimental area.
- **Split-Plot Design**: Used when one factor (e.g., cultivation equipment) cannot be fully randomized; creates hierarchy with whole plots and subplots; particularly useful when plot size or equipment constraints exist.
- **Systematic designs**: Non-randomized arrangements rarely suitable for efficacy evaluations; may only be appropriate in special cases like varietal trials on herbicide selectivity.

When designing phytosanitary product trials, the arrangement of untreated controls is critical for proper efficacy assessment. According to EPPO standards, the main purpose of untreated controls is to demonstrate adequate pest infestation, without which efficacy cannot be meaningfully evaluated. Four distinct arrangements for untreated controls exist:

- **Included controls**: The most common approach, where control plots have the same shape and size as treatment plots and are fully randomized within the experimental design. This arrangement is essential when controls will be used in statistical comparisons.

- **Imbricated controls**: Control plots are arranged systematically within the trial (between blocks or between treated plots), potentially with different dimensions than treatment plots. These observations are typically not included in statistical analyses but ensure more homogeneous distribution of untreated area effects.

- **Excluded controls**: Control plots are established outside the main trial area but in similar environmental conditions. While replication is not essential, it may be beneficial in heterogeneous environments. These observations are generally excluded from statistical analyses.

- **Adjacent controls**: Each plot is divided into two subplots, with one randomly selected to remain untreated. This approach is particularly valuable in highly heterogeneous environments but requires specialized split-plot statistical analysis.

The selection of control arrangement depends on several factors: whether the control will be included in statistical tests (requiring included controls), the degree of environmental heterogeneity (adjacent controls are preferred for high heterogeneity), and the potential for control plots to interfere with adjacent treatment plots (suggesting excluded controls when interference is likely). The trials type design is critical for the success of the study, as it ensures that the results are reliable, reproducible, and statistically valid.

1.2.2 Sampling Method and Measures Units

After defining the experimental units through the randomization design choise, the next step is to define the sampling method and the measures units. Target and crop-specific standards point out ”mode of assessment recording and measurements” fixing evaluation metrics in two ways: countable (discrete values) and measurable (continuous values) effects which must be expressed in absolute values, in other cases, frequency (incidence) and degree (severity) should be estimated and reported as affected percentage of the individual (ex. plant or plot) or as proportion within thesis and control expressed in percentage. As specified by PP 1/152 [4], classification by ranking (ordinal) and scoring (ordinal or nominal) is also contemplated. In the case of estimation, rather than count or measure, PP 1/152 reports ”The observer should be trained to make the estimations and his observations should be calibrated against a standard”. Calibration compliance with standards is ensured by GEP audits. Scoring and ranking scales examples are published on specific standards or the same PP 1/152. The lack of specific scales lets trial protocol authors define one inspired in range and intervals by the mentioned examples or other well-established ones. GEP units PP 1 assessments are produced by trained and experienced agronomists or biologists by visual inspection or laboratory analysis. The technician follows the trial protocol and related EPPO standards during assessment execution. The technician is critical for accuracy, precision, and repeatability. Sensitivity is determined by the trial protocol. It depends on expected differences and if a measure, a proportion, or a scale is used. For instance, in PP 1/93(3) [6] ”Efficacy evaluation of herbicides - Weeds in cereals - Observation on the crop”, phytotoxicity color modification could be measured, or estimated as proportion in respect to the untreated, or scored in EPPO scale as PP 1/135(4) reports, or a scientifically accepted score as the European Weed Research Society phytotoxicity damage score [2] and other ones. In general, data types must undergo the classification presented in Table 1.1

Table 1.1: Different modes of observation and types of variables

Type of Variable	Measurement	Visual Estimation	Ranking	Scoring
Binary				X
Nominal				X
Ordinal			X	X
Discrete	X	X		
Continuous limited	X	X		
Continuous not limited	X	X		

1.2.3 Statistical Analysis

The statistical analysis of trials is equally critical, providing objective assessment of treatment effects. While PP 1/152 [4] doesn’t prescribe specific analyses for all situations, it emphasizes that analysis methods should align with the experimental design and data types collected. For qunatitative variables (continuous or discrete), parametric methods based on Generalized Linear Models (GLM) are recommended, including ANOVA and regression approaches. For qualitative variables (ordinal or nominal), non-parametric methods are more appropriate. Parametric analysis assumes additivity of effects, homogeneity of variance, and normally distributed errors—

when these assumptions aren't met, data transformations or alternative approaches become necessary.

Statistical tests, particularly F-tests of orthogonal contrasts, should focus on biologically relevant comparisons specified during the design stage: untreated control versus treatments (establishing trial validity), reference products versus control (demonstrating coherence), test products versus reference (evaluating efficacy), and comparisons among test products (identifying superior treatments). For efficacy trials, EPPO suggests one-sided tests since the aim is comparing products against references or controls, with appropriate multiple comparison procedures when needed.

Through adherence to these rigorous design and analysis standards, researchers can generate reliable evidence to support phytosanitary product registration while ensuring that products demonstrate consistent efficacy across relevant agricultural conditions.

1.3 Geomatics Techniques

While the EPPO experimental design standards provide a solid foundation for conducting phytosanitary product trials, the increasing availability of digital tools and technologies offers new opportunities to enhance the quality (in the ”Quality of a mode of observation” sense [4]) and efficiency of these assessments. Digital approaches can automate data collection and analysis, improving the reproducibility of results, ultimately accelerating the development and registration of effective phytosanitary products.

To regulate the use of this kind of technologies, the EPPO published a new standard, PP 1/333(1) [1], which filled the gap in the use of digital technologies in phytosanitary product efficacy and selectivity trials. This standard provides guidelines for incorporating digital tools into trial protocols, where digital tools are intended as a combination of hardwares and softwares delivering data in a semi-automatic or automatic fashon. The digital data must respect the same quality standards of the manual ones, and the digital tools must be validated before the trial execution. Validation of digital tools should be performed by comparing the results of digital and manual assessments, demonstrating that the digital tools provide reliable and consistent results compared to manual assessments golden sample. The benchmarks for the validation depends on the type of variable. For each type of variable, the congruence between digital and manual should be evaluated with a different metric:

- **Continuous**: Coefficient of determination (R^2) higher than 0.85.
- **Ordinal and Nominal**: Cohen's kappa Coefficient (κ) higher than 0.7.
- **Binary**: Accuracy higher than 0.85

1.3.1 Geostatistics

Adoption of digital tools is not only a matter of data collection but also of data analysis. In many case analyze digital data with the same statistical methods reduce the benefits of digital data collection or simply is not possible.

One of the most compelling advantages of adopting digital approaches is the dramatic increase in the number of observations that can be collected. Unlike manual methods, which are inherently limited by human capacity and time constraints, automated and semi-automated systems can continuously gather data with minimal interruption. This greater volume of data not only improves the resolution and granularity of analysis but also significantly enhances the statistical power of hypothesis testing.

The power of a statistical test, defined as the probability of correctly rejecting the null hypothesis when it is false, directly depends on the sample size (number of observations), as noted by a classical statisticians as Fisher [10]. However effiecient technics to collect data does not garantee indipendency of samples. In other words, having a powerfull tool to collect data does not mean that we can rely on a higher amount of replications. Wheter repeated observations per experimental unit (pseudo-replications) are produced, Generalized Linear Mixed Models (GLMM) [11, 13] should be used to benefit from digital observations size enhancement. GLMMs are a powerful extension of GLMs that can account for the correlation structure of repeated observation by incorporating random effects, thus providing more accurate estimates of treatment effects and their associated uncertainty. GLMMs can also be used to model spatial and temporal autocorrelation, which is common in field trials and can significantly impact the validity of statistical inferences. Randomization of treatments within blocks is designed to minimize the impact of spatial variability, but residual spatial autocorrelation may still exist due to unmeasured environmental factors. Accounting for these correlation structures ensures that whether the randomization fails in capture environmental variability, the results are still valid and reliable.

Geostatistics encompasses a range of statistical methods aimed at analyzing spatial and spatiotemporal data. In the context of phytosanitary product trials, geostatistical methods allow for the quantification of spatial variation and the prediction of treatment effects across plots. The most prominent techniques include kriging, spatial covariates modeling, and spatial analysis of field trials with splines.

Kriging is an advanced interpolation method that provides best linear unbiased predictions (BLUP) of spatially correlated variables. By modeling the spatial covariance structure through variograms, kriging allows for accurate prediction of unmeasured locations, reducing spatial noise and enhancing the interpretation of treatment effects.

Spatial Covariates involve incorporating additional spatial information (such as soil properties or topography) into statistical models, thereby improving the accuracy of predictions. Integrating spatial covariates helps to account for environmental variability and isolate the true treatment effect.

Spatial Analysis of Field Trials with Splines leverages spline functions to model smooth spatial trends, effectively addressing spatial heterogeneity. Splines offer a flexible and efficient way to account for gradual changes across the field, complementing other geostatistical methods.

All these geostatistical techniques can be seamlessly integrated with GLMMs to provide a comprehensive analysis of spatial-temporal data, enhancing the accuracy and reliability of treatment effect estimates.

1.3.2 Photogrammetry

Photogrammetry is a geomatic technique that allows the acquisition of spatial data by processing and analyzing photographic images. Photogrammetry is a technique used to obtain reliable information about physical objects and the environment through the process of recording, measuring, and interpreting photographic images. It is widely used in various fields such as topographic mapping, architecture, engineering, manufacturing, quality control, and geology. The fundamental principle of photogrammetry is based on the geometry of image formation and the mathematical relationships between the images and the objects being photographed.

The basic principle of photogrammetry involves capturing multiple photographs of an object or scene from different perspectives. By analyzing these images, it is possible to reconstruct the three-dimensional (3D) coordinates of points on the object's surface. The key steps in photogrammetry include image acquisition, image orientation, and 3D reconstruction.

Images are typically captured using cameras mounted on various platforms such as tripods, drones, or aircraft. The quality and resolution of the images are crucial for accurate photogrammetric analysis. The images should have sufficient overlap (usually 60-80%) to ensure that common points are visible in multiple images.

Image orientation involves determining the position and orientation of the camera at the time each photograph was taken. This process is divided into two main steps: interior orientation and exterior orientation.

- **Interior Orientation:** This step involves determining the internal geometry of the camera, including the focal length, principal point, and lens distortion parameters. These parameters are typically obtained through a camera calibration process.
- **Exterior Orientation:** This step involves determining the position (X, Y, Z coordinates) and orientation (roll, pitch, yaw angles) of the camera in a global coordinate system. This is achieved by identifying and matching common points (tie points) in overlapping images and using these points to solve for the camera parameters.

Once the images are oriented, the 3D coordinates of points on the object's surface can be reconstructed using triangulation. Triangulation is a mathematical process that involves intersecting lines of sight from multiple images to determine the precise location of a point in 3D space.

Mathematically, the process can be described using the collinearity equations, which relate the image coordinates (x, y) of a point to its object coordinates (X, Y, Z) through the camera parameters:

$$x = x_0 - \frac{f \cdot (r_{11}(X - X_0) + r_{12}(Y - Y_0) + r_{13}(Z - Z_0))}{r_{31}(X - X_0) + r_{32}(Y - Y_0) + r_{33}(Z - Z_0)}$$
$$y = y_0 - \frac{f \cdot (r_{21}(X - X_0) + r_{22}(Y - Y_0) + r_{23}(Z - Z_0))}{r_{31}(X - X_0) + r_{32}(Y - Y_0) + r_{33}(Z - Z_0)}$$

where:

- (x_0, y_0) are the coordinates of the principal point in the image.
- f is the focal length of the camera.
- (X_0, Y_0, Z_0) are the coordinates of the camera position.
- r_{ij} are the elements of the rotation matrix that describes the orientation of the camera.

By solving these equations for multiple images, the 3D coordinates of the object points can be accurately determined.

1.3.3 Machine Learning

Machine Learning (ML) is a branch of artificial intelligence (AI) that focuses on the development of algorithms and models capable of learning from and making predictions or decisions based on data. Unlike traditional programming, where explicit instructions dictate the output for given inputs, ML models identify patterns and relationships within data to generate predictive outcomes. These techniques are particularly valuable when dealing with large, complex, or high-dimensional datasets, where manual analysis would be impractical or inefficient.

ML has gained substantial importance in various scientific fields, including agriculture and plant protection. Within the context of phytosanitary product efficacy evaluation, ML offers new opportunities to enhance data processing, interpretation, and decision-making by leveraging vast amounts of observational data collected during field trials. Integrating ML approaches into the framework of PP1/333 can significantly increase the robustness and accuracy of the analysis, allowing for more data-driven and automated assessments.

The primary objective of employing ML techniques in phytosanitary product trials is to improve accuracy, precision, and reproducibility while reducing manual intervention and subjective bias. Modern ML methods can analyze complex interactions between variables and predict treatment outcomes under various conditions, thereby facilitating more efficient and accurate efficacy assessments.

There are several fundamental approaches in machine learning, each suited to different types of tasks and data structures:

- **Supervised Learning**: Models are trained on labeled datasets where the input-output relationship is known. Techniques include regression, classification, and ensemble methods such as Random Forests and Gradient Boosting.
- **Unsupervised Learning**: Models identify patterns or groupings within data without labeled responses. Clustering (e.g., K-means, hierarchical clustering) and dimensionality reduction (e.g., PCA, t-SNE) are common techniques.
- **Semi-supervised Learning**: Combines a small amount of la-

beled data with a large amount of unlabeled data to improve learning accuracy.

- **Reinforcement Learning**: Agents learn by interacting with an environment and receiving feedback in the form of rewards or penalties.
- **Deep Learning**: Utilizes neural networks with multiple layers (deep architectures) to model complex relationships and patterns, particularly in image and signal processing.

ML models can also be integrated with statistical techniques, providing hybrid approaches that combine inferential statistics with predictive modeling. For example, generalized linear models (GLMs) can be enhanced with ML techniques to improve their accuracy and adaptability.

Computer vision is a subfield of ML that focuses on enabling machines to interpret and analyze visual information. In the context of phytosanitary product efficacy evaluation, computer vision methods are increasingly used for automated observation and measurement, particularly when integrated with digital imaging and photogrammetry.

The use of computer vision within PP1/333 trials significantly enhances data acquisition by enabling continuous monitoring and precise measurement of crop conditions. Techniques such as image segmentation, object detection, and texture analysis can automatically identify plant stress, disease symptoms, and pest damage. Convolutional Neural Networks (CNNs) and related architectures, including ResNet and U-Net, have shown high efficacy in analyzing complex agricultural images.

Moreover, combining computer vision with geostatistical methods allows for the spatial mapping of efficacy across field plots, generating comprehensive visual assessments that support statistical evaluations. This integrated approach maximizes the utility of both spatial and temporal data, facilitating more robust and accurate assessments while minimizing human error and labor requirements.

Chapter 2

Thesis Aims

The aim of this thesis is to investigate the potential benefits of integrating geomatics techniques in the design and analysis of phytosanitary product efficacy trials. As already discussed, the EPPO standards provide a solid foundation for conducting experimental trials, but the increasing availability of digital tools and technologies offers new opportunities to enhance the quality and efficiency of these assessments. By leveraging geomatics techniques such as photogrammetry, geostatistics, and machine learning, researchers can improve data collection, analysis, and interpretation, ultimately accelerating the development and registration of effective phytosanitary products. Throughout a study case each variable type, we will explore the opportunities and constraints of deploy geomatic techics for increase phytosanitary products effects estimation.

Chapter 3

Study Cases

3.1 Continuous Variables

3.1.1 Plant Count

3.2 Ordinal and Nominal Variables

3.2.1 Phytoxicity Score

3.3 Binary Variables

3.3.1 Embedding Spaces for Control Sample Anomaly Detection

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