Accepted manuscript

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics

Métodos de muestreo como base para conocer el estatus fitosanitario asociado a enfermedades y daños en la producción de arándanos en el trópico alto colombiano

Laura A. Valbuena-Gaona[1, 2]

Johan Sebastián Rodríguez-Arteaga[2]

Helber Enrique Balaguera-López[2]

Joaquín Guillermo Ramirez-Gil[1, 2, 3]

[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Departamento de Agronomía, Bogota (Colombia)

[2] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Laboratorio de Agrocomputación y Análisis Epidemiológico, Bogota (Colombia)

[3] Corresponding author. jgramirezg@unal.edu.co

Main phytosanitary problems of blueberry in the Colombian Highland Tropics.
Photo: J.G. Ramírez-Gil

Accepted manuscript

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

Last name: VALBUENA-GAONA / RODRÍGUEZ-ARTEAGA / BALAGUERA-LÓPEZ / RAMIREZ-GIL

Short title: SAMPLING METHODS FOR ASSESSING PHYTOSANITARY STATUS IN COLOMBIAN HIGHLAND BLUEBERRY PRODUCTION

Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

Received: 19-11-2024 Accepted: 27-02-2025 Published: 13-03-2025

ABSTRACT

Blueberry production has significantly increased globally in recent years due to its excellent nutritional quality. In Colombia, cultivated areas have expanded, along with the rise of phytosanitary problems that are still not well-characterized, nor are there reliable sampling tools for monitoring and evidence-based decision-making. This study focuses on the symptomatological characterization, identification, and determination of the incidence of the main blueberry pathologies (diseases and disorders) in Colombia, under high-altitude tropical conditions. Additionally, various sampling methods were evaluated as a basis for developing statistically valid tools for monitoring in field conditions. The research was conducted in commercial fields across nine municipalities in the Cundinamarca and Boyaca regions of Colombia. After characterizing the main diseases and disorders, the incidence was determined under field conditions, and the best sampling strategy was evaluated based on methods such as random, systematic grid-based, and stratified sampling. Sample size determination was based on the finite population method. The intensity measures evaluated incidence and severity showed that in Colombia's high-altitude tropics, the most important diseases were rust and shoot dieback, mechanical damage, and fruit dehydration as abiotic disorders. Stratified sampling yielded the best performance, showing the lowest coefficient of variation. Our findings provide the first characterization of blueberry pathologies, their significance, and a sampling method for evidence-based decision-making. This work is crucial as it establishes a methodology for identifying phytosanitary issues, proposes a robust sampling approach for field application, and emphasizes the need for ongoing detection of emerging diseases to enhance data-driven decision-making.

Additional key words: *Vaccinium corymbosum*; stratified sampling; dieback; rust; incidence; severity.

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

RESUMEN

La producción de arándanos ha aumentado significativamente a nivel mundial en los últimos años debido a su excelente calidad nutricional. En Colombia, las áreas cultivadas se han expandido, junto con el aumento de problemas fitosanitarios que aún no están bien caracterizados y para los cuales no existen herramientas de muestreo confiables para la toma de decisiones basadas en evidencia. Este estudio se centra en la caracterización sintomatológica, identificación y determinación de la incidencia de las principales patologías del arándano (enfermedades y desórdenes) en Colombia, bajo condiciones de trópico alto. Además, se evaluaron diversos métodos de muestreo como base para desarrollar herramientas estadísticamente válidas para el monitoreo en condiciones de campo. La investigación se llevó a cabo en campos comerciales de nueve municipios en las regiones de Cundinamarca y Boyacá, Colombia. Tras la caracterización de las principales enfermedades y desórdenes, se determinó su incidencia en condiciones de campo y se evaluó la mejor estrategia de muestreo basada en métodos como el aleatorio, sistemático en cuadrícula y estratificado. La determinación del tamaño de muestra se realizó mediante el método de población finita. Las medidas de intensidad evaluadas, incidencia y severidad, mostraron que en el trópico alto colombiano las enfermedades más importantes fueron la roya y la muerte regresiva de brotes, mientras que los desórdenes abióticos más relevantes fueron los daños mecánicos y la deshidratación de frutos. El muestreo estratificado presentó el mejor desempeño, con el menor coeficiente de variación. Nuestros hallazgos proporcionan la primera caracterización de las patologías del arándano, su importancia y un método de muestreo para la toma de decisiones basada en evidencia. Este trabajo es fundamental, ya que establece una metodología para identificar problemas fitosanitarios, propone un enfoque de muestreo robusto para su aplicación en campo y enfatiza la necesidad de continuar con la detección de nuevas enfermedades para mejorar la toma de decisiones basada en datos.

Palabras clave adicionales: *Vaccinium corymbosum*; muestreo estratificado; muerte descendente; roya; incidencia; severidad.

INTRODUCTION

In recent years, the global production and consumption of berries have been on the rise due to their nutritional composition, which has led to their recognition as a superfood and as an essential group of fruits within the concept of healthy eating (Silva *et al*., 2020). Among the main berries, the blueberry (*Vaccinium corymbosum* L.) stands out, being considered one of the most in-demand fruits globally due to its high antioxidant content and pleasure flavor (Rodriguez-Saona *et al*., 2019; Routray and Orsat, 2011). Central and South America are some of the most active regions in the growth of blueberry cultivation and export, including countries such as Mexico, Peru, Chile, Argentina, and Colombia, among others (Banerjee *et al*., 2020; Macha *et al*., 2023). In Colombia, blueberry production has primarily focused on the departments of Cundinamarca and Boyaca, which offer optimal agro-climatic conditions for its cultivation, although production is also reported in the departments of Antioquia and Valle del Cauca (Fischer *et al*., 2021). In general terms, one of Colombia's competitive advantages in blueberry production is the ability to produce year-round, which has allowed for a steady supply and export to countries such as the United States, Qatar, Malaysia, Singapore, Italy, and Spain (Fischer *et al*., 2021).

The expansion of blueberry cultivation areas worldwide has coincided with an increase in pre- and post-harvest phytosanitary issues, which have a significant economic impact by affecting the productivity, yield, and quality of the fruit (Rodriguez-Saona *et al*., 2019; Banerjee *et al*., 2020). Among the primary diseases affecting blueberry crops is *Botrytis cinerea* Pers., a fungus found during pre- and post-harvest stages, which is responsible for production losses exceeding 20% (Abbey *et al*., 2024). Other diseases are also reported globally, with their incidence, severity, mortality, and economic impact varying depending on several factors. Among the most important diseases are rust (*Pucciniastrum vaccinia* G. Winter) Jørst. (1952) (syn. *Naohidemyces vaccinia*), the complex of dieback (*Phomopsis* spp., *Lasiodiplodia* spp., *Neofusicoccum* spp., *Botryosphaeria* spp., *Alternaria* sp., *Pestalotiopsis* sp., and *Neopestalotiopsis* sp., and *Phomopsis* spp.), powdery mildew (*Microsphaera vaccinii*), alternaria (*Alternaria tenuissima*), anthracnose (*Colletotrichum* spp.) (Scherm *et al*., 2008; Hilário *et al*., 2020; Cline and Burrack, 2023; Ru *et al*., 2023). Abiotic factors also play a significant role, causing physiological alterations such as fruit cracking, mechanical damage, internal rotting, root asphyxiation, color changes, and chlorosis, among others (Cleves, 2021; Hou *et al*., 2024).

Therefore, it is essential to have the necessary tools not only to understand the phytosanitary status of blueberries in Colombian plantations but also to have appropriate diagnostic elements that enable the design of proper management measures. This would allow for the prevention or mitigation of damages or losses associated with biotic or abiotic problems affecting the crop (Lucas, 2011). A well-designed management practice begins with an accurate diagnosis, where recognizing the causal agent of the problem or correctly associating a symptom with the cause of the disease becomes paramount (Mohammad-Razdari *et al*., 2022; Mahaman *et al*., 2003). In particular, it is crucial to characterize the symptoms and severity under field conditions as a means to quickly recognize pathologies, forming the basis for timely decision-making, especially when other diagnostic tools are unavailable (Ramírez-Gil and Morales, 2019; Bock *et al*., 2022).

In addition to having fast and sensitive visual diagnostic methods, it is important to ensure the availability of sampling tools. Sampling not only optimizes resources by eliminating the need to assess the entire population, but also allows for the use of various methods to perform population inference processes. These methods serve as the basis for estimating the impact of a population on the productive objective, which is the crop, by inferring different variables that measure disease intensity (Madden and Hughes, 1999b). Under field conditions, population estimates can have multiple sources of variation, not only related to how the variable is quantified but also to the sampling methods used, the number of samples, the plant part to sample, crop phenology, plant location in the field, and others (Lin *et al*., 1979; Aubertot *et al*., 2004; Belan *et al*., 2021). In this context, once these sources of variation are standardized, sampling can be repeated over time, becoming a form of monitoring. Thus, monitoring forms the foundation for the development of evidence-based decision-making systems, which allow for proper management by responsibly utilizing resources and avoiding additional costs (Lagos-Ortiz *et al*., 2019).

In Colombia, an emerging country in blueberry cultivation, the phytosanitary status of this crop remains largely unknown. Currently, there is no information on the phytosanitary issues affecting blueberries, their incidence rates, associated symptoms, distribution patterns, or economic impact. Furthermore, critical decision-making tools, such as sampling strategies, remain undeveloped (Binns *et al*., 2000). Key aspects such as sampling methods, the number of plants to be sampled, and target biological units for sampling are not yet established. This gap not only limits the understanding of current pest and disease pressures but also hinders the development of effective management strategies, leaving growers without essential tools for protecting crop health and ensuring sustainable production.

While diagnosis and sampling tools are essential for proper management, they are not the only factors to consider, as the quantification and analysis of data on disease intensity directly influence decision-making regarding the most appropriate management option. This is because such analysis helps determine whether the economic cost associated with the problem is lower than the potential economic losses it could cause, and thus, whether implementing a treatment is justified (del Águila *et al*., 2015). Additionally, agricultural production is part of a heterogeneous system where variations between environmental factors (climate, soil, terrain) and ecological factors (related to biotic interactions with pests and diseases) respond to spatial and temporal patterns of differential scales (Sabtu *et al*., 2018). Therefore, the spatial and temporal analysis of phytosanitary monitoring data are tools that enable a better understanding and mitigation of the impact of diseases on blueberry crops (Rodriguez-Saona *et al*., 2019).

In general terms, decision-making scenarios differ depending on how information is collected, and which data are included in the samples (Appelhoff *et al*., 2022). Thus, depending on the nature of the problem to be sampled, the method of data acquisition may vary to adequately estimate the density or population of the issue and contribute to evidence-based decision-making processes (Madden and Hughes, 1999a; Binns *et al*., 2000; Luo *et al*., 2012). In this context, the standardization of sampling methods becomes crucial to optimize processes and ensure that the collected information is representative and consistent with the crop and the problem at hand (Madden and Hughes, 1999b; Luo *et al*., 2012; Schillaci and Schillaci, 2022). Data collected through sampling is essential for making informed decisions in crop loss assessment and pest management and the data enhances farmers' understanding of pest threats, thereby improving the quality of decision-making in crop protection practices (Hughes, 1999).

By systematically sampled collecting data on disease incidence and severity, these methods provide a foundation for informed decisions, such as the application of fungicides, implement population regulation measures, exclusion, quarantine, or cultural practices (Madden *et al*., 2007). Visual diagnostics, supported by sampling, allow for rapid evaluation of visual symptoms which are key to identifying pathogens, ensuring that management strategies are both targeted and cost-effective (Nutter *et al*., 2006). For instance, sampling methods can identify disease hotspots and, combined with visual diagnostics, improve the speed and accuracy of disease detection, ultimately supporting sustainable crop protection (Nutter, 1993). Currently, there is not only a lack of knowledge regarding the most significant diseases affecting blueberries in the high-altitude tropical regions of Colombia but also limited information on sampling methods and optimization strategies. Developing efficient diagnostic tools and field-applicable sampling techniques is crucial for improving inference capacity and practical implementation. Therefore, the objective of this study was to develop a sampling methodology to determine the main physiopathologies (diseases, disorders and damages) in blueberry crops that are adapted to the production systems in Colombia, specifically in the Colombian highland tropics. To achieve this, an initial characterization of the main physiopathological issues found in the region, associated with the observed symptoms, is presented, followed by an approach to selecting the sampling method that best suits the problems encountered.

MATERIALS AND METHODS

Study area and basic characteristics of the production systems

This study was conducted in nine blueberry-producing municipalities located in the departments of Cundinamarca and Boyaca (Colombia), situated in the high-altitude tropical cold climate region (Fig. 1). This climate is characterized by altitudes typically ranging from 2,000 to 3,000 m a.s.l., with temperatures between 12°C and 18°C. Relative humidity generally exceeds 70%, and solar radiation is intense throughout much of the year, though it can be moderated by frequent cloud cover, especially during the rainy season. Precipitation follows a bimodal pattern, with two main rainy seasons (usually from March to May and from September to November) and two drier seasons with lower rainfall (from December to February and June to August), though this distribution can vary depending on the specific region (Fig. 1).

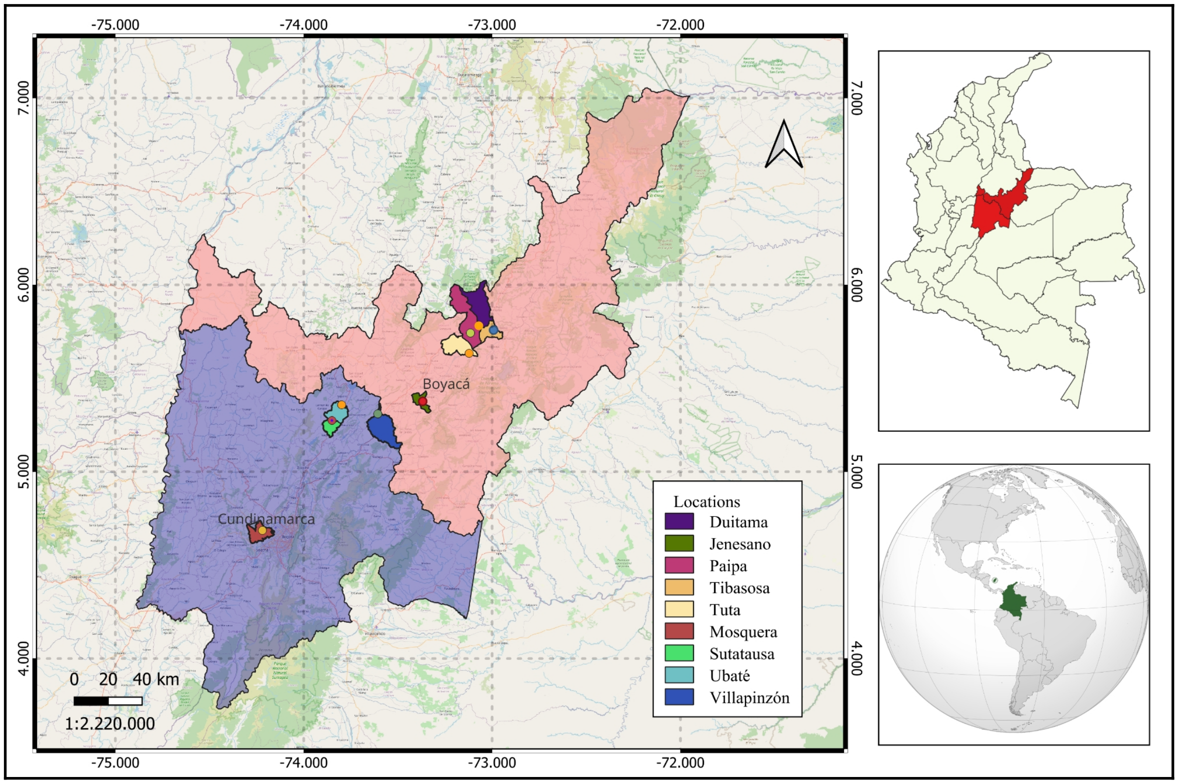

Figure 1. Blueberry sampling locations in the departments of Cundinamarca and Boyaca (Colombia).

The farms located in Cundinamarca correspond to the municipalities of Sutatausa, Ubate, Villapinzon, and Mosquera, with plant counts of 1,700, 2,000, 11,000, and 195, respectively. For the production systems in Ubate were a plastic mesh house, a macrotunnel with photoselective netting in Mosquera, plastic zenithal roof ventilation in Villapinzon, and plastic horizontal screen cover roof only in Sutatausa and Mosquera, hail protection nets were used, while Villapinzon used greenhouses and Sutatausa employed plastic covers for each row of plants. The farms located in Boyaca correspond to the municipalities of Paipa, Tuta, Tibasosa, Duitama, and Jenesano. The first two municipalities had 2,800 and 5,000 plants, while information regarding plant density were 10,000, 15,000, and 7,500 respectively. In Paipa, the plants were covered with plastic in a ridge and furrow arrayhail protection nets, while the others were under a plastic-covered under plastic-covered mesh greenhouses, and photoselective netting mesh house for Tuta (Fig. 1).

For this study, selected plots were chosen to represent the entire blueberry-producing region of the Altiplano Cundiboyacense in Colombia, where the largest high-altitude tropical cultivation areas in Colombia are located. The selection aimed to include small, medium, and large plots to maximize variability. However, we acknowledge the potential for sampling bias, particularly in securing permission from landowners for sampling access. To minimize bias, we ensured that plots belonged to different owners, were in different localities, and that all included at least the Biloxi cultivar. This approach allowed for a standardized genotype comparison while maintaining diversity across production conditions.

It is well known that disease intensity variables, such as incidence and severity, as well as their spatial and temporal dynamics, are highly influenced by climatic conditions and agronomic practices, including fungicide use, biological control tools, and cultural management strategies. Understanding this complexity, our study adopts an approach based on management strategies established by each production unit. Due to confidentiality agreements, specific details cannot be disclosed; however, in general, these strategies align with integrated management principles. They include fertilization, inoculum removal through pruning, eradication of plants in advanced disease stages, and the use of population regulation agents such as Trichoderma and Bacillus, along with both contact and curative fungicides. Regarding climatic conditions during the sampling period, precise data were unavailable. However, the study was conducted during the first half of 2024, specifically in April, May, and June, corresponding to the rainy season in the study region due to its bimodal climate system.

Symptomatological characterization of phytosanitary (diseases and disorders) issues in blueberries in the Colombian highland tropics

For this section, the observation and description of symptoms associated with the most relevant pathologies and damages in the blueberry production areas evaluated were conducted (Fig. 1). To characterize the phytosanitary issues, including those caused by microorganisms and other important alterations or disorders, a detailed review of symptoms reported in the literature was performed. These symptoms have been used as basic tools for disease identification, along with the associated damage, which was quantified using severity scales or degrees of affectation (Scherm *et al*., 2008; Moggia *et al*., 2017; Hilário *et al*., 2020; Madrid and Beaudry, 2020; Cline and Burrack, 2023; Ru *et al*., 2023). This was complemented by a previous process in which co-creation with producers and/or field technical assistants was conducted, supporting the association of symptoms with the implicated phytosanitary issue based on their local experience, previous diagnoses, and laboratory results from private Phytosanitary Diagnostic Centers. Additionally, for those symptoms where the disease and its causal agent were uncertain, tissue samples were taken from the field and sent to the plant diagnostic laboratory at the 'Universidad Nacional de Colombia', Bogotá campus, 'Clinica de plantas'.

For fieldwork, the sampling support personnel were previously trained to identify the issues present using audiovisual materials such as images and videos. For each symptom found, a photographic record was made to characterize the symptoms associated with the phytosanitary problems in the blueberry crop found in the evaluated fields, based on the previously described characterization and scales.

Sampling methods evaluated to determine the population inference of phytosanitary (diseases and disorders) problems in blueberries

The methodology used to calculate the sample size (*n*) was based on the formula for finite populations with normal distribution and unknown population parameters (Eq. 1) (Cochran, 1997). The adjustment in each field was made according to the total size of the population of plants planted (*N*: number of plants planted), the maximum accepted estimation error (10-20%), and the confidence level (80-90%, based on the specific *Z* values), thus allowing an accurate estimation of the parameters of interest (Cochran, 1997). Three types of sampling were carried out in each production system: i) simple random, ii) systematic on grid, and iii) proportional stratified. In each one, the number of sampling points previously established was maintained. The grid sampling was evaluated according to the dimensions of the farm to have symmetry in the points, guaranteeing the same distances in the spatial

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

dimensions *x* and *y*. On the other hand, stratified sampling was performed by assigning the main extract criterion according to the particularity of each production system with parameters of greater relevance such as phenology or age, slope of the farm and/or variety. For the particular case of the distribution of the points in the strata, a random sub-sampling was followed, otherwise, there was no variable to determine a sub-stratum. For the simple random sampling, it was defined based on a random criterion to define each of the points to be evaluated, represented as a plant, simulating these based on the flat xy coordinates, according to the number of plants, and using a random function

$$n=\frac{N*Z_{\alpha}^{2}*p*q}{e^{2}*(N-1)+Z_{\alpha}^{2}*p*q} \tag{1}$$

where: *n*=sample size, N=total population size. Z_{α}=critical value of the standard normal distribution, related to the desired confidence level, *p*=proportion of the characteristic under study in the population (0.5), *q*=complementary proportion to *p* (0.5) and *e*=admissible margin of error.

For all plots, the determination of the sample size (*n*) started from the total population (*N*, as previously defined). A balance was sought between field practicality and statistical inference capability, using a 90% confidence level (*Z* value of 1.65) and a margin of error of 10%. Based on these parameters, and considering the convergence of nnn as per the Central Limit Theorem (Cochran, 1997), the optimal sample size for most plots ranged between 66 and 68 plants. An exception was observed in the Mosquera plot, where the lower total number of plants led to a reduced sample size. The final sample sizes (*n*) per plot were as follows: Sutatausa: 66, Ubate: 66, Villapinzon: 68, Mosquera: 51, Paipa: 67, Tuta: 68, Tibasosa: 68, Duitama: 68 and Jenesano: 68. This approach ensured consistency in sampling methodology while adapting to the specific characteristics of each plot.

Variables evaluated in the field and data analysis

The incidence and severity of the identified problems were systematically evaluated in each plot, using continuous and discrete values according to predefined values of severity and incidence. The severity of diseases in blueberries assessed in the field was measured by quantifying the proportion of infected tissue. This was determined through a visual estimation of the affected area, where the presence of disease symptoms was observed and categorized (Bock *et al*., 2022; García-León *et al*., 2024). The accuracy and reliability of this method were ensured by using diagrammatic scales that had been rigorously evaluated and calibrated. These scales served as a reference for translating visual assessments into quantitative values, offering a standardized representation of the actual proportion of infected tissue in the plant (Bock *et al*., 2022; García-León *et al*., 2024). We aligned field observations with validated diagrammatic scales, to provide a consistent and accurate approach for assessing disease severity, ensuring that visual estimates reflected real conditions observed in the infected tissue. The incidence of the disease was determined based on the severity levels evaluated at each sampling point. The presence of the disease was defined as severity levels greater than 1 on the previously validated scale. If a sampling point exhibited symptoms according to these criteria, it was classified as positive for the disease. In contrast, the absence of symptoms was associated with level 0 on the severity scale, indicating no evidence of the disease at that point. To analyze the results, descriptive statistical methods were employed to derive insights at two distinct levels: regional (high tropics) and local (municipality). Each analysis was tailored to the sampling methods used, ensuring that comparisons were meaningful and context-specific.

To identify behavior patterns between locations associated with the intensity measures of the phytosanitary (diseases and disorders) problems found, a clustering analysis were performed. The most informative variables were identified using a Principal Component Analysis (PCA) and graphed in loading plots to identify relations between variables. Then, the clustering analysis was development using *k*-means method, the optimal number of clusters were determined using the elbow method. Both analyses were performed with the scikit-learn library (Pedregosa *et al*., 2011) in python programming language, using the VisualStudio development environment.

To evaluate the performance of each sampling method, the coefficient of variation (CV) was chosen as the primary metric (Brown, 1998). This statistical measure, defined as the ratio of the standard deviation to the mean, is particularly effective in assessing variability, offering a straightforward yet highly informative way to compare consistency across methods (Brown, 1998; Shechtman, 2013). A lower CV value indicates less variability in the data, which reflects a more reliable sampling method (Brown, 1998; Shechtman, 2013). The simplicity of the CV makes it a powerful tool for determining the best sampling approach, as it provides a clear and objective benchmark for evaluating data precision and consistency (Shechtman, 2013; Schillaci and Schillaci, 2022). Sampling methods with the lowest CV were considered the most robust, as they demonstrated the greatest reliability in capturing representative data across diverse settings (Binns and Nyrop, 1992; Brown, 1998; Shechtman, 2013; Schillaci and Schillaci, 2022). Using this approach, our approach established a rigorous framework for evaluating sampling methodologies, ensuring that the selected methods were efficient, consistent, and adaptable to varying geographical and environmental conditions (Binns and Nyrop, 1992).

Mesoscale spatial analysis associated with principal diseases problems of blueberries

To approximate the spatial patterns of the main diseases affecting blueberries in the high tropics, an analysis was conducted to identify the distribution and variability of these diseases across different plots. This approach aimed to determine whether the diseases exhibited random, clustered, or uniform patterns within the evaluated areas (Plantegenest *et al*., 2007). The spatial analysis was carried out based on a meso-scale analysis at the regional level. Once the best sampling method was selected, incidence and severity were calculated for each farm. An analysis of the spatial distribution of the main phytosanitary problems associated with diseases found in the sampled fields was performed. This was done using the kernel density interpolation algorithm, which is a method that estimates the probability function of the distribution based on points of presences. For the calculation of densities, the Tophat kernel (Eq. 2) was used because, unlike others, it does not smooth the distribution by assigning the same weights to all points and allows us to identify the foci found in the fields without an adjustment to a particular distribution (Nelson and Boots, 2008; Liu *et al*., 2012). For the calculation of the probability distribution, the incidence and severity values were used as weights to add a magnitude factor for the pathology or damage evaluated. These analyses were performed using the KernelDensity tool of the scikit-learn library (Pedregosa *et al*., 2011). Additionally, through a cross-validation process, the bandwidth value was optimized according to the data collected in the field to avoid overestimation, for which values between 0.1 and 1 were evaluated with the model selection tool of the same library using the freely available programming language Python

$$K\left(u\right)=\frac{1}{2h}\,\text{if}\vee u\vee h\text{else}\,0 \qquad (2)$$

where: K=kernel, u=value of point and h=bandwidth.

RESULTS AND DISCUSSION

Symptomatological characterization of the major diseases and disorders in blueberries in the high tropics

Under the field conditions evaluated in this study (Fig. 1) and within the time frame during which the assessment was conducted, the most significant diseases and disorders identified were: botrytis blight, rust, dieback, alternaria, sooty mold, mechanical damage, anthocyanescence, and fruit dehydration (Fig. 2). Additionally, other pathologies and disorders were observed, but due to their low relevance or inconsistent symptomatology, they were not included in the scope of this study. This focused approach ensured that the analysis prioritized the most impactful issues affecting blueberry crops within the defined evaluation parameters.

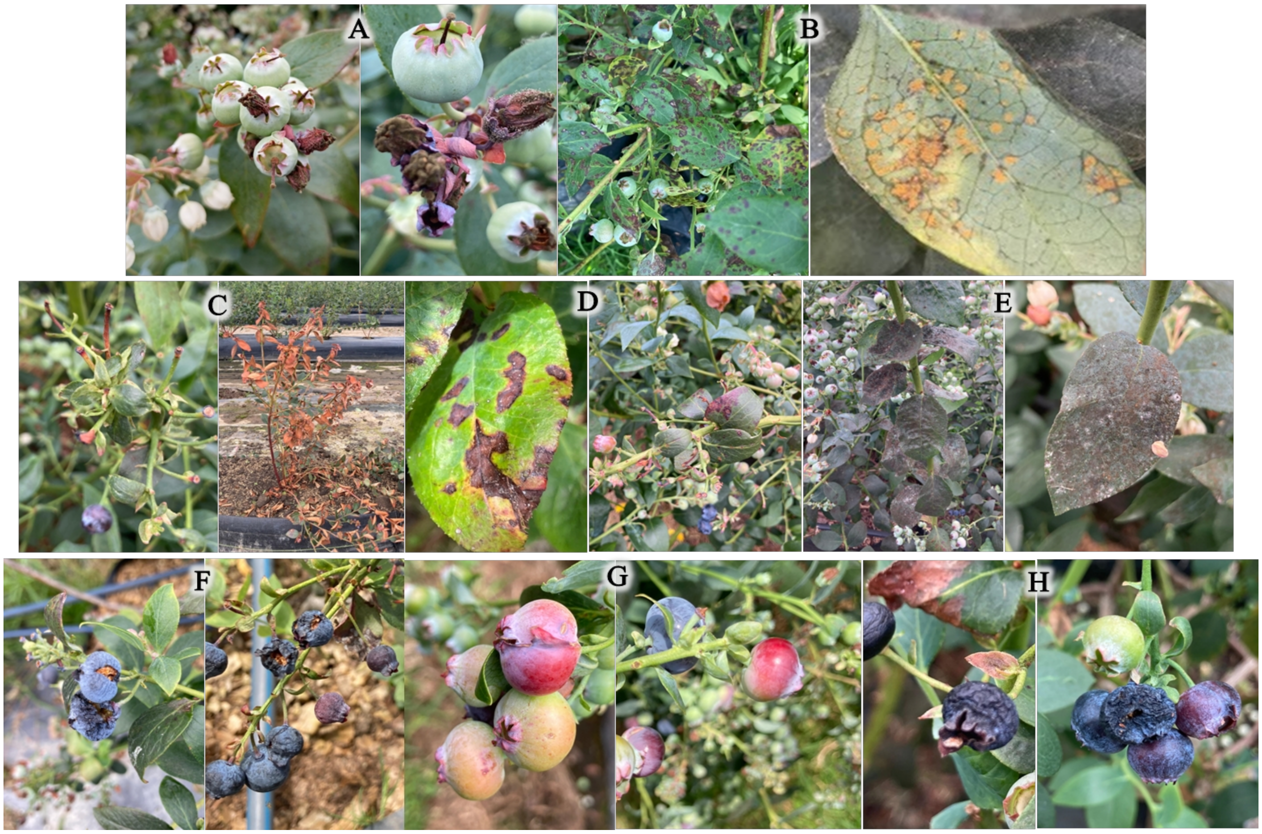

Figure 2. Symptomatology found on blueberry plants in the sampled plots. (A) Botrytis blight. (B) Rust. (C) Dieback. (D) Alternaria. (E) Sooty mold. (F) Mechanical damage (Birds and rodents feeding). (G) Fruit decoloration (Anthocyanesence). (H) Fruit dehydration.

The symptoms associated with botrytis blight (*B. cinerea*) were the stunting of the flower buds, which tend to turn violet to dark colors, normally covered by gray mold associated with the signs of the pathogen (Fig. 2A). For rust, caused by *Pucciniastrum* sp., orange or yellow pustules were identified on the underside of leaves and in more advanced stages premature defoliation of the plant (Fig. 2B). Dieback is caused by various causal agents and is characterized by necrosis from the terminal tops to the base, as well as fruit dehydration (Fig. 2C). Alternaria (*Alternaria* sp.) was identified by the presence of necrotic spots surrounded by a chlorotic halo, although it can also present fruit rot in advanced stages (Fig. 2D), and the presence of sooty mold on the leaf blades characterized by a mycelial growth that covers the leaves with dark colorations (Fig. 2E).

Mechanical damage caused by birds and rodents was also identified, which manifested as fruit bites and leaf lesions (Fig. 2F), fruit discoloration or anthocyanescence associated with partial or total changes in fruit color (Fig. 2G), and fruit dehydration, which showed loss of turgor and was not associated with downward death (Fig. 2H).

Measures of intensity of diseases and disorders associated with blueberries in the high tropics: Incidence and severity

Botrytis blight was found in all the farms sampled except for Mosquera, and when using stratified sampling it was also found in Sutatausa. The highest incidence of the disease was found in Tibasosa with systematic on grid sampling, and Tuta and Ubate locations when using random sampling. Paipa presented the lowest incidence for all three sampling methods, followed by Ubate with stratified sampling (Fig. 3A). Rust was reported in all farms sampled except for Mosquera Paipa and Sutatausa, and in Ubate it was not found when using random sampling. The highest incidences were observed in Tuta and Ubate with stratified and systematic on grid sampling. The lowest incidences were observed in Villapinzon (Fig. 3B). Dieback was not found in the plots sampled in Paipa, Sutatausa, and Ubate using systematic on grid sampling. The highest incidences were observed in Mosquera, and the lowest incidences were observed in Ubate, and when using random sampling in Villapinzon (Fig. 3C). Alternaria was not found in the field located in the municipality of Mosquera, in Tibasosa it was not found when random sampling was used, in Sutatausa when stratified sampling was used, and in the municipalities of Paipa, Tuta, and Ubate it was only found when random sampling was used. In the municipality of Villapinzon the highest incidence of the disease was reported when random sampling was used, and in Paipa the lowest incidence was reported (Fig. 3D). Sooty mold was only found in Villapinzon and Duitama. The highest incidence was observed with random sampling in Villapinzon and the lowest incidence was observed in Duitama with systematic on grid sampling (Fig. 3E).

Regarding physiological disorders, no mechanical damage associated with feeding animals such as birds or rodents was found in Paipa, as well as in Tuta, Ubate and Villapinzon using random sampling, and Duitama with stratified sampling. The greatest damage was observed in Tibasosa and Duitama using systematic on grid sampling, and the least damage was observed in Mosquera and Villapinzon (Fig. 3F). Anthocyanescence was observed in all the sampled plots. Sutatausa showed the highest incidences using stratified sampling, as did Mosquera with stratified and systematic on grid sampling, Tuta with random sampling, and Ubate with random and stratified sampling, while the lowest incidences were observed using systematic on grid sampling in Ubate and Tibasosa (Fig. 3G). All fields sampled showed fruit dehydration. The highest incidence of the physiological disorders was observed in Tuta with random sampling and Ubate with stratified sampling, and the lowest incidence was observed in Paipa, and when using stratified and systematic on grid sampling in Tuta (Fig. 3H).

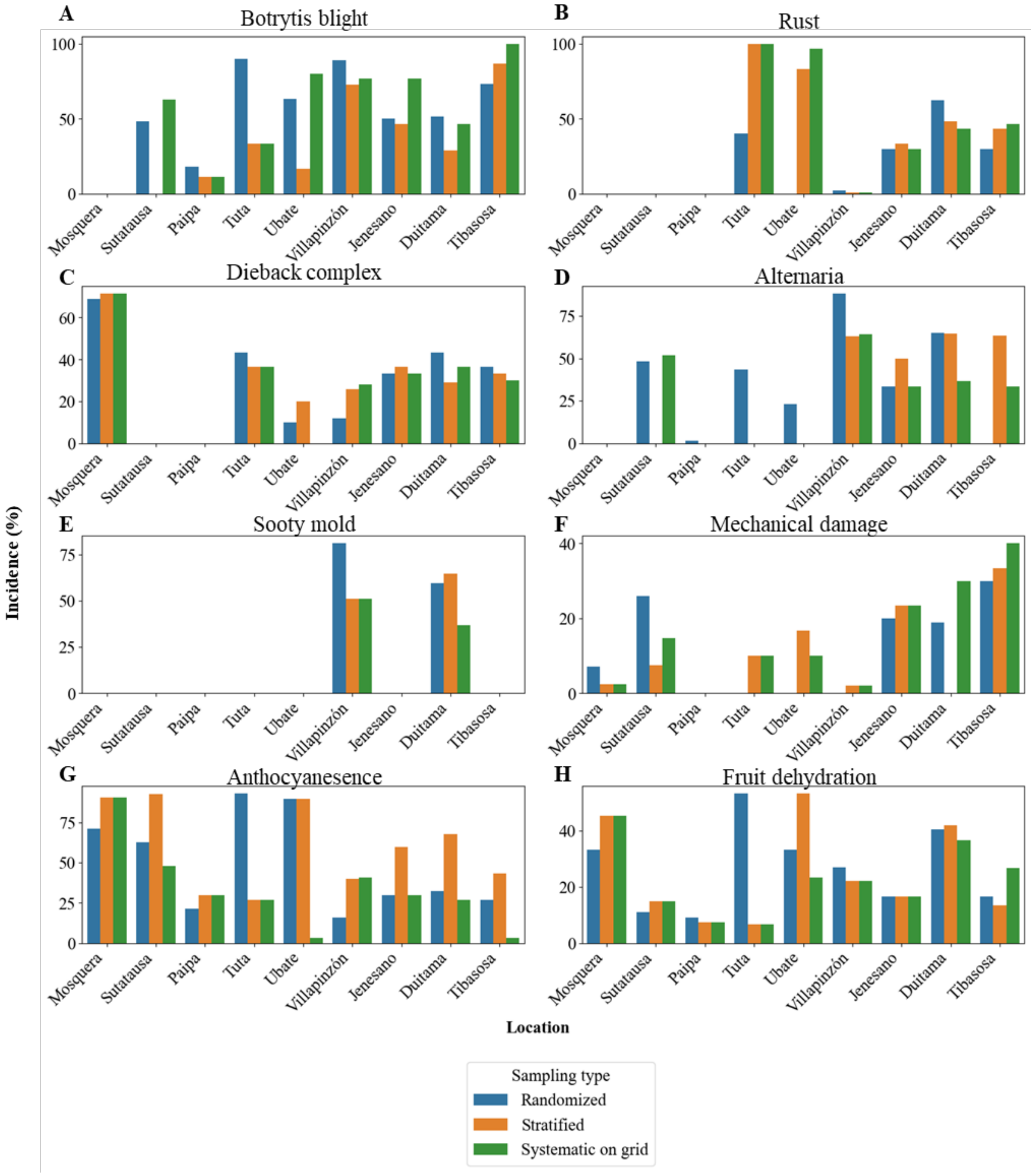

Figure 3. Incidence of problems found in blueberry plants grown in different locations. (A) Botrytis blight. (B) Rust. (C) Dieback. (D) Alternaria. (E) Sooty mold. (F) Mechanical damage (Birds and rodents feeding). (G) Fruit decoloration (Anthocyanesence). (H) Fruit dehydration.

For botrytis blight, the highest severity was observed with random sampling in the municipality of Duitama. In this same municipality, the lowest severities were observed with the other sampling methods, as well as in the municipality of Sutatausa, when using systematic on grid sampling and stratified sampling in Paipa and random sampling in Tuta and Ubate (Fig. 4A). Rust presented the highest severity values in the municipality of Tuta with stratified and systematic on grid sampling, while the lowest severities were observed in Villapinzon and Jenesano (Fig. 4B). Dieback showed the highest severity in Tuta with random sampling, and Villapinzon with stratified and systematic on grid sampling. The lowest severities were observed in Ubate, Tuta with stratified and systematic on grid sampling, and Duitama with systematic on grid sampling (Fig. 4C). Alternaria presented greater severity in Villapinzon and Duitama when using random sampling. The lowest severity was observed in Ubate and Tibasosa with systematic on grid sampling (Fig. 4D). Systematic on grid sampling presented the highest and lowest sooty mold values for the municipalities of Villapinzon and Duitama, respectively (Fig. 4E).

In the case of disorders associated with mechanical damage due to bird and rodent feeding occurred with greater severity in the municipalities of Duitama and Tibasosa with systematic on grid and random sampling, respectively. The lowest severity was observed in Mosquera, Ubate, Villapinzon, Jenesano, Sutatausa with random sampling, Duitama with random sampling, and Tibasosa with systematic on grid sampling (Fig. 4F). The highest anthocyanin severity was observed in Villapinzon, with random sampling in Paipa, Tuta, and Ubate, stratified in Jenesano, and random and stratified in Duitama. The lowest severities were observed in Mosquera, Sutatausa, Tibasosa, with stratified and systematic on grid sampling in Paipa, Tuta, and Ubate, random and systematic on grid sampling in Jenesano, and systematic on grid sampling in Duitama (Fig. 4G). For fruit dehydration, the highest severity was observed in Villapinzon, and the lowest severity values were found in Mosquera, Paipa, Sutatausa with random and stratified sampling, Tuta with stratified and systematic on grid sampling, Ubate with stratified sampling, and Tibasosa with random and systematic on grid sampling (Fig. 4H).

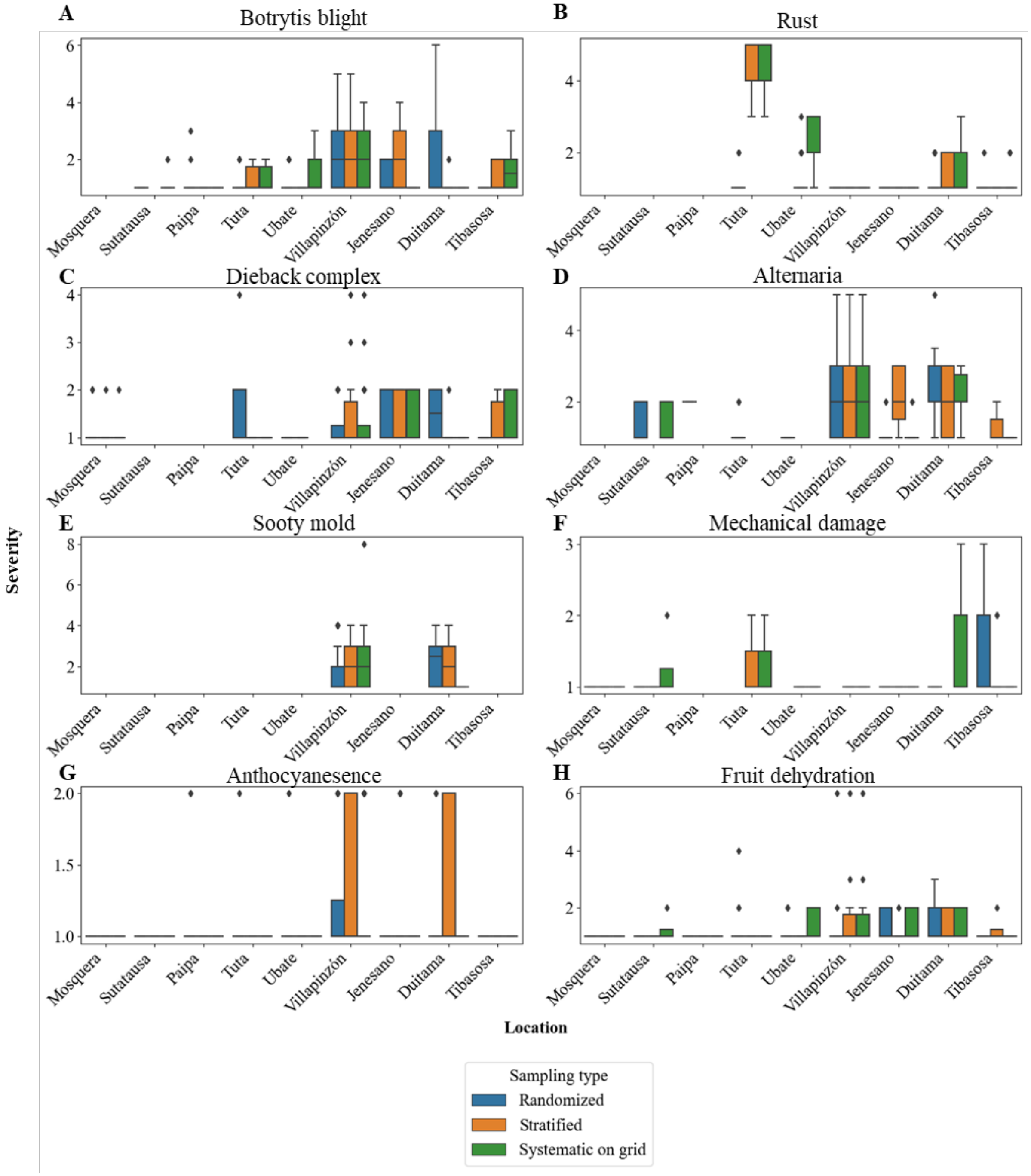

Figure 4. Severity of problems found in blueberry plants grown in different locations. (A) Botrytis blight. (B) Rust. (C) Dieback. (D) Alternaria. (E) Sooty mold. (F) Mechanical damage (Birds and rodents feeding). (G) Fruit decoloration (Anthocyanesence). (H) Fruit dehydration.

PCA shows anthocyanesence, dieback complex and fruit dehydration-mechanical damage, as important features for PC1-PC2, respectively (Fig. 5A). Clustering analysis shows that Tibasosa and Jenesano tend to have similar incidence behavior of the main phytosanitary problems (Fig. 5B), regarding the incidence values, both locations had high incidence of botritys blight and alternaria, absence of sooty mold, and medium to low incidence of the other problems (Fig. 3). Paipa, Sutatausa and Villapinzon are clustered together (Fig. 5B), possibly related to the absence of sooty mold, rust (very low incidence for Villapinzon) and low incidence of fruit dehydration, but the behavior regarding the other phytosanitary problems is less similar (Fig. 3). The cluster Tuta, Ubate, Duitama, and Mosquera (Fig. 5B) shared similar behavior in anthocyanescence and fruit dehydration incidence, and sooty mold that is absence in all locations except Duitama, for the other problems found, Tuta, Ubate and Duitama shared similar incidence values (Fig. 3).

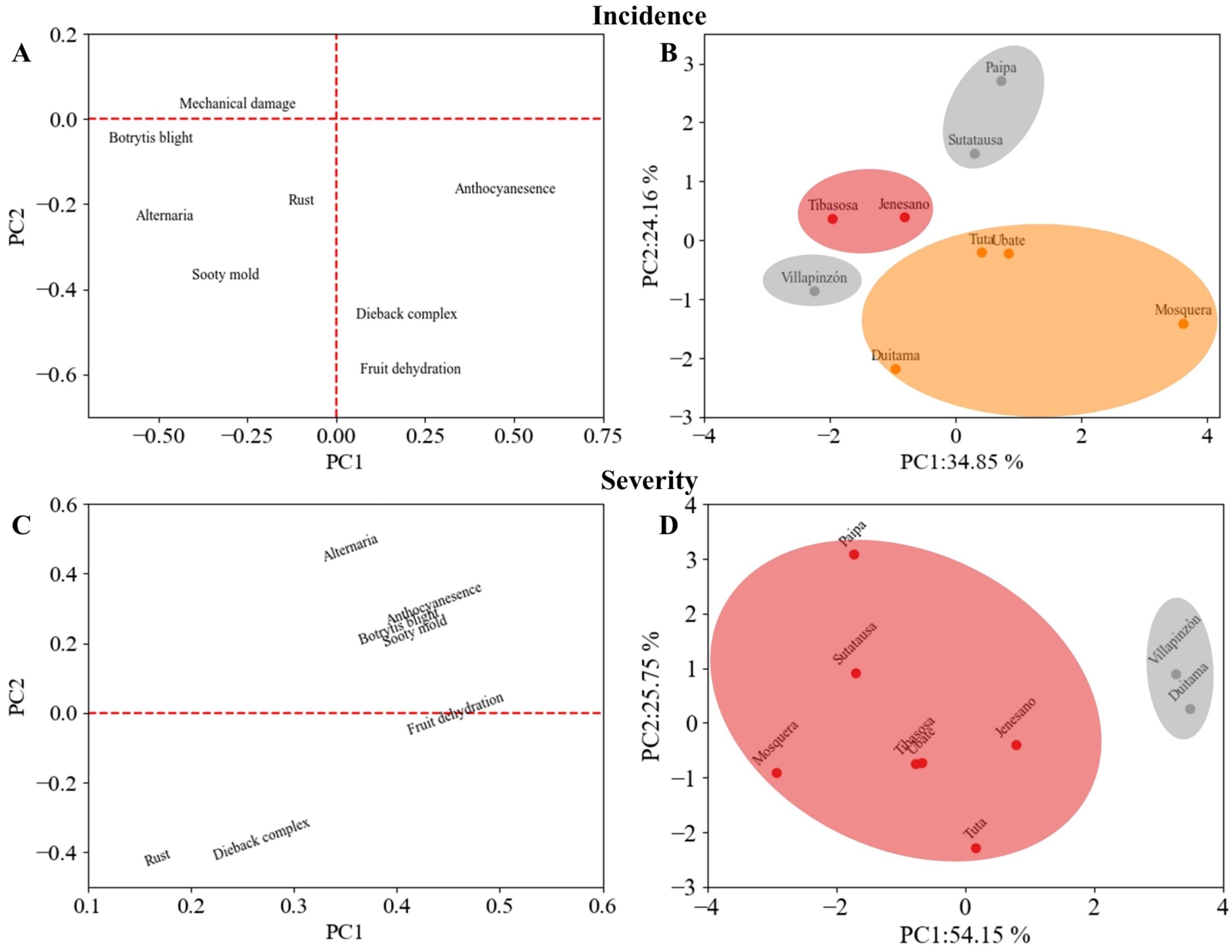

Figure 5. Principal components analysis (PCA) loading plot for PC1 and PC2. (A) Severity. (C) Incidence. Clustering analysis by location. (B) Severity. (D) Incidence. Percentage values represent total variability that are explained by each PC.

For the severity analysis, alternaria, anthocyanesence, botrytis blight and sooty mold are grouped together as the main importance variables for both components, suggesting that they assume the major variability (Fig. 5C). Two clusters were defined, isolating Villapinzon and Duitama (Fig. 5D). Based on the severity values, the main phytosanitary problems that could define the clusters are antocyanescence, due to its high severity compared to the other locations, and sooty mold, because the problem is just found in both locations. For botrytis blight and alternaria, the severity is slightly higher in these locations (Fig. 3). PCA analysis for incidence and severity shows that variables such as alternaria, botrytis blight and sooty mold are related, as well as the fruit dehydration and dieback complex (Fig. 5A and C).

Evaluation of the type of sampling as a basis for estimating variables to measure the intensity of the main diseases in blueberries

The fields with the highest CV were those located in the municipalities of Villapinzon, Jenesano, Duitama and Tibasosa for the three types of sampling. When analyzing by municipality, the best sampling method is stratified and grid for Mosquera, Paipa, and Tuta with values of 1.05%, 0.9%, and 2% for both respectively. Random sampling presented better results in Tausa and Duitama with a CV of 1.23% and 8.2% respectively. Stratified sampling showed the least variation in the municipalities of Ubate, Jenesano, and Tibasosa with values of 0.24%, 12.39%, and 13.33%, respectively. Gridded sampling shows better results in Villapinzón with a CV of 7.22% (Fig. 6A). Stratified sampling shows less variation in the High Tropic with a CV of 64.72% (Fig. 6B).

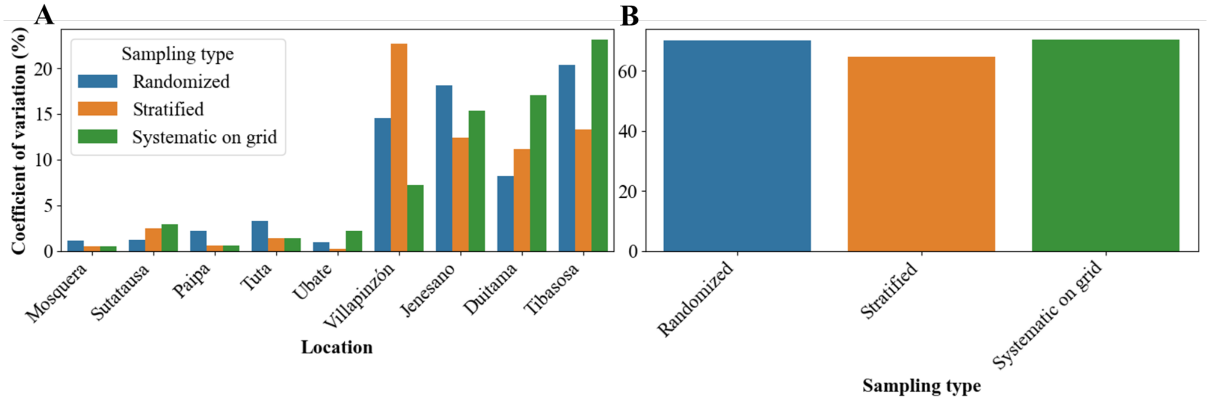

Figure 6. Coefficients of variation determined for each of the three sampling methods as a metric of reliability, precision, and consistency in the population inference of phytosanitary problems in blueberries through sampling inference. (A) Mean coefficient of variation of all phytosanitary problems evaluated by location. (B) Total sums mean of the coefficient of variation for all phytosanitary problems evaluated by sampling method.

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

Spatial analysis of phytosanitary problems of blueberries in the high tropical region

Based on the kernel density model fit and subsequent interpolation to understand the spatial distribution of major blueberry diseases in Colombia's high tropical region, a robust representation of spatial behavior was achieved. Specifically, cross-validation determined bandwidth values of 0.9 for the incidence of *Botrytis cinerea* and both the incidence and severity of *Alternaria tenuissima* and sooty mold. For other evaluated phytosanitary issues, the optimal bandwidth was 0.8. These results relate to the bias-variance trade-off in estimation; bandwidth values approaching 1 may result in oversmoothing, potentially obscuring critical data structure details (underfitting).

At the mesoscale level within the Cundiboyacense plateau's production region in Colombia, spatial relationship analyses indicate that botrytis blight and rust manifest in clusters concerning both incidence and severity. These clusters are primarily located in Boyaca and the northeastern part of Cundinamarca, where most sampled farms are situated. An exception is the farm in Mosquera, located in the southwest of Cundinamarca, which exhibits low disease intensity levels (Fig. 7A, B, C, and D). The dieback complex shows variability; incidence rates are low across many evaluated plots, except in Mosquera, where a cluster is present. However, severity levels display a clustered pattern throughout most of the assessed region, indicating that this pathology is of significant concern despite low incidence rates, suggesting a localized behavior with high severity values (Fig. 7E and F). Alternaria and sooty mold exhibit similar distributions, with clusters in Boyaca and northeastern Cundinamarca. These clusters are more pronounced than those observed for botrytis blight and rust but have lower severity values, indicating widespread distribution with a potentially lesser impact on the affected host area (severity) (Fig. 7G, H, I, and J).

This study offers the first comprehensive approximation of the phytosanitary status associated with blueberry pathologies under high-altitude tropical conditions in Colombia. We conducted a detailed symptomatological characterization and analyzed its implications for specific diseases. Based on this characterization, field sampling was carried out to assess the incidence and severity of biotic and abiotic pathologies in key blueberry-growing regions. The most notable diseases identified include rust (*P. vaccinia*), botrytis blight (*B. cinerea*), the dieback complex, alternaria (*Alternaria* spp.), and sooty mold, among others. Among these, rust and dieback were of particular concern due to their severity and potential for significant yield loss and fruit quality impact. The phytosanitary status of blueberries in Colombia partially aligns with other producing regions, where diseases like botrytis and rust vary in importance based on local conditions, management practices, and genotypic differences (Abbey *et al.*, 2024). Notably, the high severity of dieback has been identified as one of the diseases with the greatest potential impact and risk to the crop (Hilário *et al.*, 2020; Ru *et al.*, 2023).

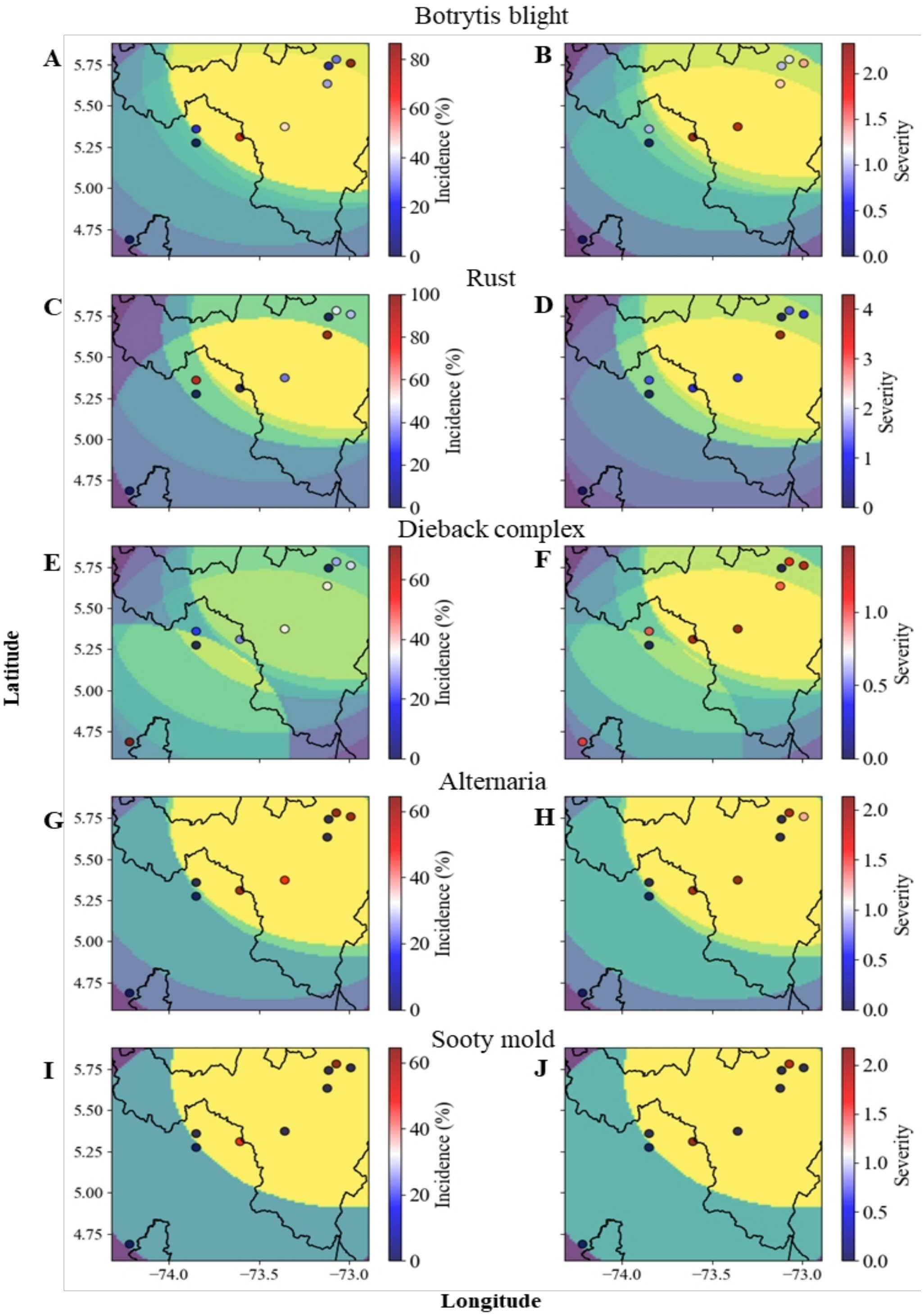

Figure 7. Kernel density maps illustrating the spatial distribution of incidence (A, C, E, G, I) and severity (B, D, F, H, J) of the main phytosanitary issues affecting blueberry cultivation in the

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

high tropics. These maps enable visualization of concentration and dispersion patterns of diseases, aiding in the identification of areas with higher phytosanitary risk.

Mechanical and other abiotic factors causing damage or losses were initially identified under field conditions. This aspect is crucial, as blueberries are highly susceptible to damage and loss of visual qualities. Consumers prioritize visual attributes such as the absence of defects and uniform blue color when assessing quality. Regarding, the mechanical damage caused by birds, rodents, and handling practices was also identified as a major factor impacting both yield and quality. In addition, fruit dehydration and anthocyanin development were found to have substantial effects, not only reducing fruit count but also compromising fruit quality (Fan *et al*., 2017; Cleves, 2021; Hou *et al*., 2024).

Despite these significant findings, several limitations were identified that should be addressed in future research. One of the primary challenges was the lack of precise diagnostic methods available in the field based on a polyphasic approach (Ramírez-Gil and Morales, 2019). Although symptom-based characterization provided valuable insights, confirming the specific causal agents remains a challenge. Current diagnostic methods are limited in their ability to provide the accuracy needed for effective management. Multifaceted, more advanced diagnostic techniques, such as nested, multiplex, quantitative, bio, and magnetic-capture hybridization PCR techniques, post and isothermal amplification methods, DNA and RNA based probe development, and next-generation sequencing, are needed to precisely identify pathogens and their impact on blueberry crops (Donoso and Valenzuela, 2018; Hariharan and Prasannath, 2021). Additionally, while symptoms on aerial parts of the plant were useful for identifying the presence of diseases, this approach does not account for pathogens that may affect hidden structures like roots and stems, vascular bundles among others (de Silva *et al*., 1999; Holland *et al*., 2014; Cline and Burrack, 2023). Future sampling protocols should include this tissue analysis to ensure a more comprehensive assessment of phytosanitary status of blueberries.

In addition, a deeper understanding of environmental and ecological factors are critical for enhancing disease management strategies in this region. Expanding the scope of the research to include other geographic regions, as well as investigating the economic and environmental impacts of various disease management practices, will be essential for ensuring the sustainable production of blueberries. It is essential to characterize the phytosanitary status of blueberries in Colombia's high-altitude tropics under different climatic conditions, as key modulators of disease populations. Additionally, understanding specific management practices is crucial to assessing their impact on disease intensity variables, identifying potential effects, and improving control strategies for more effective disease management.

In terms of disease monitoring, stratified sampling proved to be the most effective method for estimating sampling parameters. This method demonstrated lower bias, greater precision, and higher reproducibility compared to other sampling approaches (Singh and Mangat, 1996). Stratified sampling allowed for a more nuanced understanding of disease distribution across the field, which is critical for developing accurate and sustainable disease management strategies (Binns *et al*., 2000). The results highlight the importance of stratified sampling as a key tool for phytosanitary monitoring, offering reliable data for decision-making in disease management interventions.

In the realm of sampling and monitoring for population inference of phytosanitary issues across various crops, the implementation of robust methodologies remains nascent. This deficiency often leads to suboptimal data quality and significant biases when estimating the spatial and temporal behavior of plant diseases (Madden and Hughes, 1999b; Luo *et al*., 2012). Consequently, there is an imperative need to enhance existing sampling tools. In this sense, our approach presents advances in the need to have sampling methods that manage to reduce bias and increase the precision of population inference, however, in these processes, it is necessary to improve and optimize other basic elements associated with sampling theory. While stratified sampling provided a reliable estimate of disease parameters, the sample size (n), the effect of patch scale on disease intensity, phenological stages of the crops, spatiotemporal heterogeneity of disease, among other variations may be more carefully considered (Madden and Hughes, 1999a; Turechek and Madden, 2001; Luo *et al*., 2012). Seasonal variations, particularly during the rainy and dry seasons, could influence disease dynamics significantly. Climate variations in high-altitude tropical regions, where these blueberries are cultivated, can result in varying microclimates that affect the incidence and severity of phytosanitary issues (Ramírez-Gil and Morales-Osorio, 2018). The need for adaptive sampling strategies that account for these climatic shifts is imperative for accurate disease forecasting and management.

Our spatial analysis provides a mesoscale approximation of potential macro-regions exhibiting higher intensities of major blueberry diseases under high tropical conditions. This approach enables the identification of spatial patterns potentially associated with climatic conditions and management practices. Such information is valuable not only for regional epidemiological surveillance within the sector (Plantegenest *et al*., 2007; Nelson and Boots, 2008) but also as a tool for prioritizing specific disease management practices at the local level. In addition, knowledge of problem aggregation

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

patterns can contribute to the design and implementation of management strategies to optimize production, improve resource use, and minimize environmental impact (Savary *et al*., 2006; Nelson and Boots, 2008). In that context, future research should explore alternatives like the use of remote sensing technologies, such as drones and satellite imagery, which could help identify disease hotspots and monitor large blueberry production areas in real time. These technologies could complement traditional field diagnostics and improve early detection of disease outbreaks.

The development of this study not only identified a gap in knowledge regarding the recognition and epidemiological parameters (incidence and severity) associated with the phytosanitary status of blueberries but also highlighted a significant lack of understanding in this crop and others concerning the validation, implementation, and tools for disease inference in plants based on probability sampling method. This knowledge is essential for making informed decisions in plant health management (Hughes, 1999). Most studies in this field were conducted in the 1990s and primarily focused on validating existing methods, proposing variations of them or through simulations (Lin *et al*., 1979; Madden *et al*., 1996; Madden and Hughes, 1999b; Nutter *et al*., 2006). However, few have assessed these methods under real field conditions, limiting their applicability in practical scenarios. There is a growing need to provide reliable tools and validated approaches to companies in the agricultural sector, which increasingly require high-quality data to incorporate into their decision-making processes. Addressing these gaps will enhance disease monitoring, improve inference accuracy, and contribute to the development of more effective management strategies.

CONCLUSION

In Colombian high-altitude tropics, the most important diseases and disorders identified were botrytis blight, rust, dieback, alternaria, sooty mold, mechanical damage, anthocyanescence, and fruit dehydration. Stratified sampling yielded the best performance, showing the lowest coefficient of variation. Our findings present the first characterization of blueberry pathologies, their significance, and a systematic sampling method for evidence-based decision-making, offering the first approximation of the phytosanitary status of diseases in blueberry crops in Colombia. Future research could include the effect of patch scale on disease intensity, climatic variability, crop phenology, among other variables that affect the spatio-temporal distribution of the disease in order to obtain accurate inferences that allow correct decision making, including new tools and technologies that facilitate these processes.

Conflict of interests: The authors disclaim no conflict of financial interests or personal relationships that could have appeared to influence this work.

Author's contributions: L.A.V.G.: Methodology, Investigation, Data curation, Writing – original draft, Validation, Writing – review & editing. J.S.R.A.: Conceptualization, Writing – original draft. H.E.B.L.: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. J.G.R.G.: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Validation, Writing – review & editing.

Funding statement: This research received no external funding, but two of the members (H.E.B.L. and J.G.R.G) are faculty members affiliated with the Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Bogotá campus, where the institution supports research by providing salaries for their dedicated time to conducting research.

Open source research data repository: We have shared the data and code to ensure transparency and reproducibility of our work. We kindly request that if these resources are used for any academic purpose, the original article be cited: Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. The data of sampling and the codes used in this work, could be found in the free repository: https://github.com/agrocompuepidemlab/Sampling-methods-for-diseases-and-damage-in-blueberry-production, in order to guaranty reproducibility and transparency of the results shown in this work.

BIBLIOGRAPHIC REFERENCES

Abbey, J., D. Percival, L. Jaakola, and S.K. Asiedu. 2024. Efficacy, persistence and residue levels of fungicides for Botrytis control in wild blueberry. Crop Prot. 179, 106633. Doi: https://doi.org/10.1016/j.cropro.2024.106633

Appelhoff, S., R. Hertwig, and B. Spitzer. 2022. Control over sampling boosts numerical evidence processing in human decisions from experience. Cereb. Cortex 33(1), 207. Doi: https://doi.org/10.1093/cercor/bhac062

Aubertot, J.N., J.J. Schott, A. Penaud, H. Brun, and T. Doré. 2004. Methods for sampling and assessment in relation to the spatial pattern of phoma stem canker (*Leptosphaeria maculans*) in oilseed rape. Eur. J. Plant Pathol. 110(2), 183-192. Doi: https://doi.org/10.1023/B:EJPP.0000015359.61910.3b

Banerjee, S., G.A. Nayik, J. Kour, and N. Nazir. 2020. Blueberries. pp. 593-614. In: Nayik G.A. and A. Gull (eds.). Antioxidants in fruits: properties and health benefits. Springer, Singapore. Doi: https://doi.org/10.1007/978-981-15-7285-2_31

Belan, L.L., L.L. Belan, L.S. Satiro, W.B. Moraes, W.C. de Jesus Junior, and W.B. Moraes. 2021. A comparison of sampling methods to accurately estimate the incidence of leaf rust on conilon coffee. Australas. Plant Pathol. 50(6), 729-737. Doi: https://doi.org/10.1007/s13313-021-00823-y

Binns, M.R. and J.P. Nyrop. 1992. Sampling insect populations for the purpose of IPM decision making. Ann. Rev. Entomol. 37, 427-453. Doi: https://doi.org/10.1146/annurev.en.37.010192.002235

Binns, M.R., J.P. Nyrop, and W. van der Werf. 2000. Sampling and monitoring in crop protection: The theoretical basis for developing practical decision guides. CABI, Wallingford, UK.

Bock, C.H., K.S. Chiang, and E.M. Del Ponte. 2022. Plant disease severity estimated visually: a century of research, best practices, and opportunities for improving methods and practices to maximize accuracy. Trop. Plant Pathol. 47(1), 25-42. Doi: https://doi.org/10.1007/s40858-021-00439-z

Brown, C.E. 1998. Coefficient of variation. pp. 155-157. In: Brown, C.E. (ed.). Applied multivariate statistics in geohydrology and related sciences. Springer, Berlin. Doi: https://doi.org/10.1007/978-3-642-80328-4_13

Cleves, J.A. 2021. Fundamentos técnicos del cultivo del arándano (*Vaccinium corymbosum* L.) en la región central de Colombia. Editorial UPTC, Tunja, Colombia. Doi: https://doi.org/10.19053/9789586604895

Cline, W.O. and H.J. Burrack. 2023. Diseases and insects of economic importance affecting blueberry fruit in North Carolina. Acta Hortic. 1357, 229-234. Doi: https://doi.org/10.17660/ActaHortic.2023.1357.33

Cochran, W.G. 1997. Sampling techniques. 3rd ed. John Wiley and Sons, New York, NY.

de Silva, A., K. Patterson, C. Rothrock, and R. McNew. 1999. Phytophthora root rot of blueberry increases with frequency of flooding. HortScience 34(4), 693-695. Doi: https://doi.org/10.21273/HORTSCI.34.4.693

del Águila, I.M., J. Cañadas, and S. Túnez. 2015. Decision making models embedded into a web-based tool for assessing pest infestation risk. Biosyst. Eng. 133, 102-115. Doi: https://doi.org/10.1016/j.biosystemseng.2015.03.006

Donoso, A. and S. Valenzuela. 2018. In-field molecular diagnosis of plant pathogens: recent trends and future perspectives. Plant Pathol. 67(7), 1451-1461. Doi: https://doi.org/10.1111/ppa.12859

Fan, S., C. Li, W. Huang, and L. Chen. 2017. Detection of blueberry internal bruising over time using NIR hyperspectral reflectance imaging with optimum wavelengths. Postharvest Biol. Technol. 134, 55-66. Doi: https://doi.org/10.1016/j.postharvbio.2017.08.012

Fischer, G., D. Miranda, S. Magnitskiy, H.E. Balaguera-López, and Z. Molano (eds.). 2021. Avances en el cultivo de las berries en el trópico. SCCH, Bogota. Doi: https://doi.org/10.17584/IBerries

García-León, E., J.M. Tovar-Pedraza, L.A. Valbuena-Gaona, V.H. Aguilar-Pérez, K.Y. Leyva-Madrigal, G.A. Mora-Romero, and J. G. Ramírez-Gil. 2024. Identification of the causal agent of Guar leaf blight and development of a semi-automated method to quantify disease severity. Trop. Plant Pathol. 49, 825-837. Doi: https://doi.org/10.1007/s40858-024-00676-y

Hariharan, G. and K. Prasannath. 2021. Recent advances in molecular diagnostics of fungal plant pathogens: a mini review. Front. Cell. Infect. Microbiol. 10, 600234. Doi: https://doi.org/10.3389/fcimb.2020.600234

Hilário, S., A. Lopes, L. Santos, and A. Alves. 2020. Botryosphaeriaceae species associated with blueberry stem blight and dieback in the Centre Region of Portugal. Eur. J. Plant Pathol. 156(1), 31-44. Doi: https://doi.org/10.1007/s10658-019-01860-6

Holland, R.M., R.S.C. Christiano, E. Gamliel-Atinsky, and H. Scherm. 2014. Distribution of *Xylella fastidiosa* in blueberry stem and root sections in relation to disease severity in the field. Plant Dis. 98(4), 443-447. Doi: https://doi.org/10.1094/PDIS-06-13-0680-RE

Hou, J., B. Park, C. Li, and X. Wang. 2024. A multiscale computation study on bruise susceptibility of blueberries from mechanical impact. Postharvest Biol. Technol. 208, 112660. Doi: https://doi.org/10.1016/j.postharvbio.2023.112660

Hughes, G. 1999. Sampling for decision making in crop loss assessment and pest management: introduction. Phytopathology 89(11), 1080-1083. Doi: https://doi.org/10.1094/PHYTO.1999.89.11.1080

Lagos-Ortiz, K., J. Medina-Moreira, A. Alarcón-Salvatierra, M.F. Morán, J. del Cioppo-Morstadt, and R. Valencia-García. 2019. Decision support system for the control and monitoring of crops. pp. 20-28. In: Valencia-García, R., G. Alcaraz-Mármol, J. del Cioppo-Morstadt, N. Vera-Lucio, and M. Bucaram-Leverone (eds.). ICT for Agriculture and Environment. Springer, Cham, Switzerland. Doi: https://doi.org/10.1007/978-3-030-10728-4_3

Lin, C.S., G. Poushinsky, and M. Mauer. 1979. An examination of five sampling methods under random and clustered disease distributions using simulation. Can. J. Plant Sci. 59(1), 121-130. Doi: https://doi.org/10.4141/cjps79-017

Liu, S., W. Liu, X. Zhou, and P. Gong. 2012. Kernel-based parametric analytical model of source intensity distributions in lithographic tools. Appl. Opt. 51(10), 1479-1486. Doi: https://doi.org/10.1364/AO.51.001479

Valbuena-Gaona, L.A. J.S. Rodríguez-Arteaga, H.E. Balaguera-López, and J.G. Ramirez-Gil. 2025. Sampling methods as a basis for assessing the phytosanitary status of diseases and damage in blueberry production in the Colombian highland tropics. Revista Colombiana de Ciencias Hortícolas 19(1), e18515. Doi: https://doi.org/10.17584/rcch.2025v19i1.18515

Lucas, J.A. 2011. Advances in plant disease and pest management. J. Agric. Sci. 149(1), 91-114. Doi: https://doi.org/10.1017/S0021859610000997

Luo, W., S. Pietravalle, S. Parnell, F. van den Bosch, T.R. Gottwald, M.S. Irey, and S.R. Parker. 2012. An improved regulatory sampling method for mapping and representing plant disease from a limited number of samples. Epidemics 4(2), 68-77. Doi: https://doi.org/10.1016/j.epidem.2012.02.001

Macha, R., F.C. Navarro Soto, A. Ramírez Ríos, and E.A. Alfaro Paredes. 2023. International market concentration of fresh blueberries in the period 2001-2020. Hum. Soc. Sci. Commun. 10(1), 1-12. Doi: https://doi.org/10.1057/s41599-023-02455-7

Madden, L.V. and G. Hughes. 1999a. An effective sample size for predicting plant disease incidence in a spatial hierarchy. Phytopathology 89(9), 770-781. Doi: https://doi.org/10.1094/PHYTO.1999.89.9.770

Madden, L.V. and G. Hughes. 1999b. Sampling for plant disease incidence. Phytopathology 89(11), 1088-1103. Doi: https://doi.org/10.1094/PHYTO.1999.89.11.1088

Madden, L.V., G. Hughes, and G.P. Munkvold. 1996. Plant disease incidence: inverse sampling, sequential sampling, and confidence intervals when observed mean incidence is zero. Crop Prot. 15, 621-632. Doi: https://doi.org/10.1016/0261-2194(96)00025-7

Madden, L.V., G. Hughes, and F. van den Bosch. 2007. The study of plant disease epidemics. APS Press, St. Paul, MN.

Madrid, M. and R. Beaudry. 2020. Small fruits: raspberries, blackberries, blueberries. pp. 335-346. In: Gil, M.I. and R. Beaudry (eds.), Controlled and modified atmospheres for fresh and fresh-cut produce. Academic Press, London. Doi: https://doi.org/10.1016/B978-0-12-804599-2.00020-X

Mahaman, B.D., H.C. Passam, A.B. Sideridis, and C.P. Yialouris. 2003. DIARES-IPM: a diagnostic advisory rule-based expert system for integrated pest management in *Solanaceous* crop systems. Agric. Syst. 76(3), 1119-1135. Doi: https://doi.org/10.1016/S0308-521X(02)00187-7

Moggia, C., J. Graell, I. Lara, G. González, and G. A. Lobos. 2017. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged highbush blueberries (*Vaccinium corymbosum* L.). Front. Plant Sci. 8, 535. Doi: https://doi.org/10.3389/fpls.2017.00535

Mohammad-Razdari, A., D. Rousseau, A. Bakhshipour, S. Taylor, J. Poveda, and H. Kiani. 2022. Recent advances in E-monitoring of plant diseases. Biosens. Bioelectron. 201, 113953. Doi: https://doi.org/10.1016/j.bios.2021.113953

Nelson, T.A. and B. Boots. 2008. Detecting spatial hot spots in landscape ecology. Ecography 31(5), 556-566. Doi: https://doi.org/10.1111/j.0906-7590.2008.05548.x

Nutter, F.W. 1993. Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. Phytopathology 83(8), 806. Doi: https://doi.org/10.1094/Phyto-83-806

Nutter, F.W., P.D. Esker, and R.A.C. Netto. 2006. Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data. Eur. J. Plant Pathol. 115(1), 95-103. Doi: https://doi.org/10.1007/s10658-005-1230-z

Pedregosa, F., G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, and É. Duchesnay. 2011. Scikit-learn: machine learning in Python. J. Mach. Learn. Res. 12(85), 2825-2830.

Plantegenest, M., C. Le May, and F. Fabre. 2007. Landscape epidemiology of plant diseases. J. R. Soc. Interface, 4(16), 963-972. Doi: https://doi.org/10.1098/rsif.2007.1114

Ramírez-Gil, J.G. and J.G. Morales. 2019. Polyphasic identification of preharvest pathologies and disorders in avocado cv. Hass. Agron. Colomb. 37(3), 213-227. Doi: https://doi.org/10.15446/agron.colomb.v37n3.78528

Ramírez-Gil, J.G. and J.G. Morales-Osorio. 2018. Microbial dynamics in the soil and presence of the avocado wilt complex in plots cultivated with avocado cv. Hass under ENSO phenomena (El Niño – La Niña). Sci. Hortic. 240, 273-280. Doi: https://doi.org/10.1016/j.scienta.2018.06.047

Rodriguez-Saona, C., C. Vincent, and R. Isaacs. 2019. Blueberry IPM: past successes and future challenges. Ann. Rev. Entomol. 64, 95-114. Doi: https://doi.org/10.1146/annurev-ento-011118-112147

Routray, W. and V. Orsat. 2011. Blueberries and their anthocyanins: factors affecting biosynthesis and properties. Compr. Rev. Food Sci. Food Saf. 10(6), 303-320. Doi: https://doi.org/10.1111/j.1541-4337.2011.00164.x

Ru, S., S. Ding, J.E. Oliver, and A. Amodu. 2023. A review of Botryosphaeria stem blight disease of blueberry from the perspective of plant breeding. Agriculture 13(1), 1. Doi: https://doi.org/10.3390/agriculture13010100

Sabtu, N.M., N.H. Idris, and M.H.I. Ishak. 2018. The role of geospatial in plant pests and diseases: an overview. IOP Conf. Ser.: Earth Environ. Sci. 169(1), 012013. Doi: https://doi.org/10.1088/1755-1315/169/1/012013

Savary, S., B. Mille, B. Rolland, and P. Lucas. 2006. Patterns and management of crop multiple pathosystems. Eur. J. Plant Pathol. 115(1), 123-138. Doi: https://doi.org/10.1007/s10658-005-0651-z

Scherm, H., A.T. Savelle, P.M. Brannen, and G. Krewer. 2008. Occurrence and prevalence of foliar diseases on blueberry in Georgia. Plant Health Prog. 9(1), 18. Doi: https://doi.org/10.1094/PHP-2008-0421-01-RS

Schillaci, M.A. and M.E. Schillaci. 2022. Estimating the population variance, standard deviation, and coefficient of variation: sample size and accuracy. J. Hum. Evol. 171, 103230. Doi: https://doi.org/10.1016/j.jhevol.2022.103230

Shechtman, O. 2013. The coefficient of variation as an index of measurement reliability. pp. 39-49. In: Doi, S.A.R. and G.M. Williams (eds.). Methods of clinical epidemiology. Springer, Berlin. Doi: https://doi.org/10.1007/978-3-642-37131-8_4

Silva, S., E.M. Costa, M. Veiga, R.M. Morais, C. Calhau, and M. Pintado. 2020. Health promoting properties of blueberries: A review. Crit. Rev. Food Sci. Nutr. 60(2), 181-200. Doi: https://doi.org/10.1080/10408398.2018.1518895

Singh, R. and N.S. Mangat. 1996. Stratified sampling. pp. 102-144. In: Singh, R. and N.S. Mangat (eds.). Elements of survey sampling. Springer, Dordrecht, Netherlands. Doi: https://doi.org/10.1007/978-94-017-1404-4_5

Turechek, W.W. and L.V. Madden. 2001. Effect of scale on plant disease incidence and heterogeneity in a spatial hierarchy. Ecol. Model. 144(1), 77-95. Doi: https://doi.org/10.1016/S0304-3800(01)00350-7