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*Review*

# Quantification of virus syndrome in chili peppers

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**One of the most important problems to produce chili crops is the presence of diseases caused by pathogen agents, such as viruses, therefore, there is a substantial necessity to better predict the behavior of the diseases of these crops, determining a more precise quantification of the disease's syndrome that allows the investigators to evaluate better practices, from handling to the experimental level and will permit producers to take opportunistic corrective action thereby, reducing production loses and increasing the quality of the crop. This review discussed methods that have been used for the quantification of disease in plants, specifically for chili peppers crops, thereby, suggesting a better alternative for the quantification of the disease' syndromes in regards to this crop. The result of these reflections indicates that most methods used for quantification are based on visual assessments, discarding differences of data between distinctive evaluators. These methods generate subjective results.**

**Key words**: Quantification, plant diseases, severity, syndrome, viruses.

## INTRODUCTION

Since the inception of agriculture, the study of plant diseases has been of great interest to humanity. Plant diseases are caused by pathogens, such as fungi, oomycetes, bacteria, nematodes, viruses that cause serious economic losses to both agricultural and horti-cultural crops (Anderson et al., 2004; Gamliel, 2008; Leuven, 2006; Vlugt, 2006). Handling of diseases in chili cultivation has gained importance in the world. All chili peppers belong to the genus *Capsicum* of the Solanaceae family of plants (Ochoa-Alejo and Ramírez-Malagón, 2001). *Capsicum annuum* L. is the most popular chili species grown worldwide (Mahasuk et al., 2009; Moscone et al., 2007). They are an indispensable ingredient employed for food preparation in the world and an important product utilized in the pharmaceutical, food, cosmetic and poultry industries (Jin et al., 2009). The main producers of chili are China, Mexico, Turkey and USA. The export value of this species represents US $2,811,590,000 worldwide. In Mexico, its production represents US $576,690,000 (Valadez-Bustos et al., 2009). As with other crops, the production of chili pepper is affected by biotic and abiotic factors that reduce its crop quality and yield (Berrocal and Chaverri, 2009; Mondino, 2008; Ochoa-Alejo and Ramírez-Malagón, 2001; Polishchuk et al., 2006; Pscheidt, 2003; Valadez-Bustos et al., 2009). Viruses are responsible for causing severe losses in pepper crop production (Lee et al., 2009). In particular, the group of viruses known as geminiviruses is principally found in tropical and subtropical zones. These viruses are generally transmitted by insect vectors to a great variety of mono and dicotyledonous plants (Cerkauskas, 2004; Fernández et al., 2009). Some of the symptoms caused by Geminiviruses include: rolling up of the leaf, wrinkles, yellow mosaic, midget growth and chlorosis and generally, a notable reduction in yield (Toruño, 2005). Geminiviruses are characterized by their circular single stranded DNA

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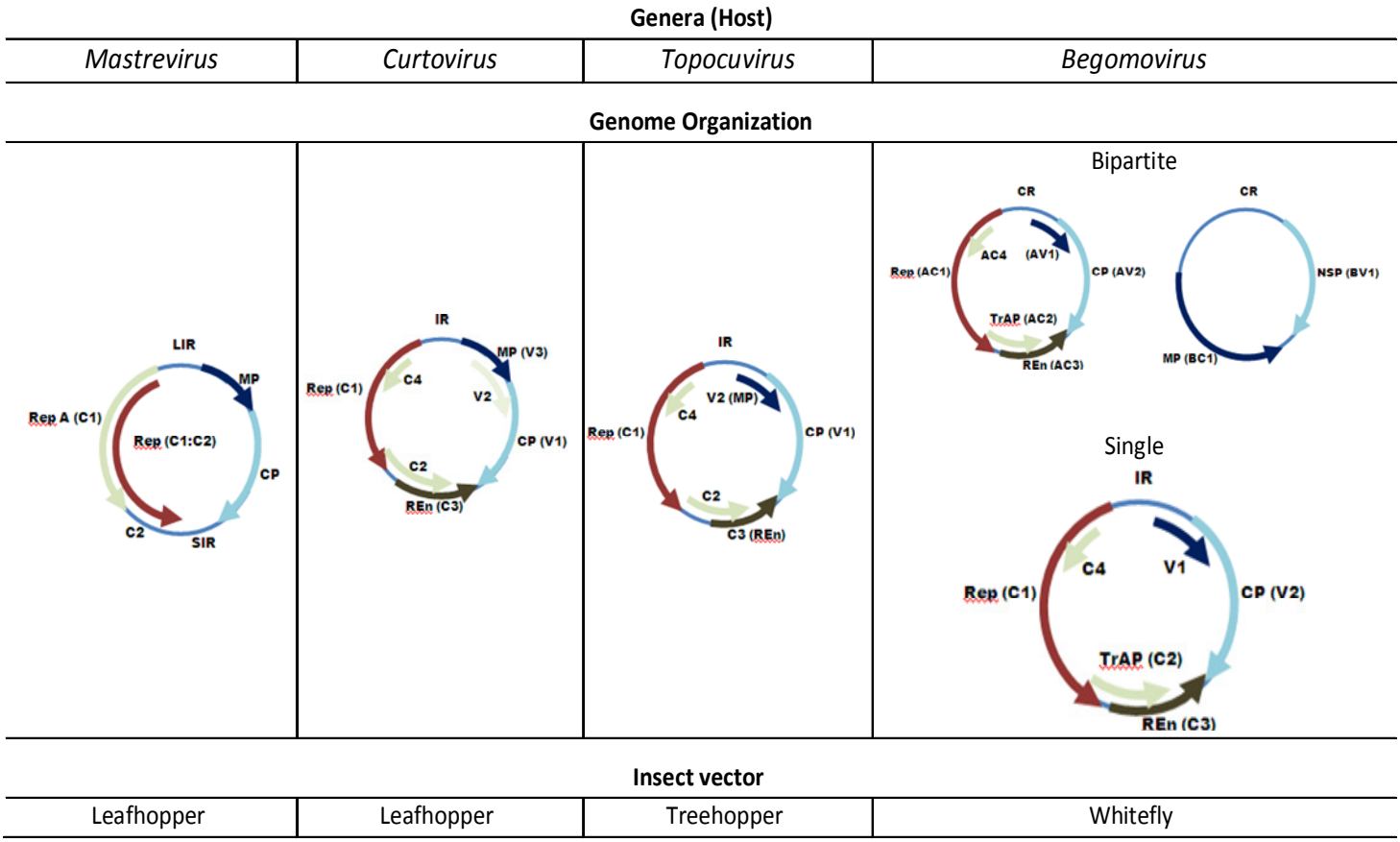


**Figure 1.** Classification of geminiviruses.

genomes, encapsidated in twinned icosahedra particles (Anaya-Lopez et al., 2003; Fauquet et al., 2008; Fernández et al., 2009; García-Cano et al., 2008; Godínez-Hernández et al., 2001; Gutierrez, 2000; Krupovic et. al., 2009; Morales and Anderson, 2001; Mubin et al., 2007; Mugiira et al., 2008; Pietersen et al., 2008; Tahir et al., 2009; Zelada, 2009; Zúñiga and Ramírez, 2002). Based on the insect vector, genome organization and host range geminiviruses are classified into four genera (Figure 1): Begomovirus, Mastrevirus, Curtovirus and Topocuvirus (Agrios, 2005; Bananej et al., 2009; Briddon et al., 2008; Carrillo-Tripp et al., 2006; Chatterjee et al., 2007; Fazeli et al., 2008; Ha et al., 2008; He et al., 2009; Hernández-Zepeda et al., 2007; Huang et al., 2006; Hull, 2004; Hussain et al., 2009; Ito et al., 2009; Kumar et al., 2008; León et al., 2004; Nawaz-ul-Rehman and Fauquet, 2009; Rentería et al., 2008; Rivera and Vega-Arreguín, 2001; Rojas, 2004; Sakata et al., 2008; Singh et al., 2009; Thresh, 2006; Yang et al., 2007).

Hybrids and varieties of *C. annuum* are severely attacked by begomoviruses in Mexico and Central America (Thresh, 2006). Begomoviruses have a monopartite or bipartite genome, transmitted by whitefly vector (*Bemisia tabaci*) and infect dicotyledonous plants (Dong et al., 2007; Kumar et al., 2008; Martínez, 2008; Ueda et al., 2008). Two of the most important begomoviruses affecting chili plants are *Pepper Huasteco yellow vein* virus *(PHYVV)* and *Pepper golden mosaic* virus *(PepGVV)* (Thresh, 2006).

The objective of this analysis was to understand the most common methods to quantify plant diseases and propose new approaches, specifically in the syndromes of diseases which are caused by viruses affecting the growth of chili peppers. There is crucial urgency to propose new forms of quantification of disease in chili pepper which is supported by traditional and compu-tational vision methods in order to decrease the sub-jectivity in the evaluation and therefore, permitting better prediction of the diseases' behavior in order to take corrective action and reduce crop losses.

## QUANTIFICATION OF PLANT DISEASES

Disease assessment is defined as the process of quantitatively measuring disease intensity. In plant pathology, there are two basic and distinct populations that can be quantitatively assessed; the pathogen population and the disease population (Nutter et al., 2006).

Disease assessment is directly related to the stage at which it is being developed. Factors such as temperature, moisture and crop plant resistance will influence the final disease level more than the initial inoculum (Cooke et al., 2006; Lovell et al., 2004). In some cases, infected plants only show slight symptoms or do not show symptoms (asymptomatic or latent infection). The symptoms of disease can be confused with pathogens but can be caused by other problems (Salazar, 1986). Furthermore, the combined infection of several viruses can change the symptom's expression, as it is the case with geminiviruses.

Quantification of the disease syndrome in plants consists of a set of measurements carried out at a predetermined point in time. There are several factors or variables that need to be considered to conduct the quantification, for example, the presence of local symptoms such as chlorosis lesions, systemic symptoms such as lesions fund on almost the entire plant and other agents such as physical, chemical and biological that are capable of inducing similar symptoms as of those produced by viruses (Hull, 2004). Before the quantification can be carried out, it is necessary to ascertain the presence of pathogens already present in the plant. Accurate detection and identification of plant pathogens are fundamental to plant pathogen diagnostics and thus, plant disease management. The specific limitations of culture-based morphological techniques to adequately identify plant pathogens have led to the development of culture-independent molecular approaches. In the last two decades, many different serological and nucleic acid-based techniques have been developed for the detection and identification of plant pathogens. Some of these techniques also permit reliable quantification of the target pathogen and supply the information that is required to estimate risks with respect to disease development, spread of the inoculum and economic losses (Leuven, 2006).

Detection methods are classified as biochemical, microscopy, immunology, nucleic acid hybridization and other traditional methods such as identification by visual inspection *in situ* or *in vitro* in pure cultures by microscopic examination (Fox, 1997). Some advantages and disadvantages of these methods are described in Table 1.

Conventional methods to detect plant pathogens have often relied on interpretation of symptoms, biochemical or morphological identification, usually following isolation and culturing of the organism *in vitro* and sometimes, on further characterization based on pathogenicity tests. Although, these methods are fundamental to diagnostics, the accuracy and reliability of these methods largely depend on skilled taxonomical expertise. In addition, diagnosis requiring a culturing step is time consuming and labor intensive. Furthermore, quantification based on these culturing techniques is considered relatively inaccurate and unreliable. Finally, these techniques rely on the ability of the organism to be cultured *in vitro*. This latter aspect is a considerable limitation since possibly less than 1% of the microorganisms in an environmental sample may be cultured *in vitro* (Leuven, 2006).

In contrast, more recently developed methods that are based on molecular approaches are increasingly being used to detect and identify plant pathogens. These include immunological (or serological) and nucleic acid-based techniques. Compared to conventional assays, these techniques are more suitable for routine analyses since they are generally faster, more specific, more sensitive and more accurate and can be performed and interpreted by personnel with no taxonomical expertise. In addition, since no culturing step is required, these techniques are equally suitable for the detection of culturable as well as non-culturable microorganisms. Many different molecular assays have been described for the detection and identification of pathogens, each requiring its own protocol, equipment and expertise (Leuven, 2006).

Once the pathogens are identified, quantification methods of disease syndromes are used with respect to plant disease management, especially quantification of a pathogen upon its detection and identification; is an important aspect as it can be used to estimate potential risks regarding disease development, spread of the inoculum and economic losses. Apart from this potential, it provides the information required to take appropriate management decisions. Nevertheless, several studies have shown that by extensive optimization of PCR conditions, quantification in endpoint analysis-based polymerase chain reaction (PCR) assays can be perfo-rmed. More recently, the introduction of real-time PCR technology, which is characterized by on-line measurement of amplicons as they accumulate during each cycle has improved and simplified methods for PCR-based quantification. Currently, in plant pathology, real-time PCR is the most reliable culture-independent technique to quantify the presence of specific pathogens as well as for the quantification of disease progress (Leuven, 2006). While nucleic acid-based assays provide an excellent opportunity for rapid and precise detection, currently their success largely depends on well-equipped laboratory facilities (Leuven, 2006).

Numerous qPCR (Quantitative polymerase chain reaction) methods have been developed and used for detection and quantification of plant pathogens and for disease diagnostics (Berruyer et al., 2006; Li et al., 2008).

Quantification of disease progression is desirable for numerous reasons including evaluating control strategies and predicting future levels of disease (Madden, 1980). Epidemiological models can be classified in several ways. For convenience, Kranz and Royle (1978) classified them into three types such as descriptive, predictive and conceptual, according to their main objective (Maanen and Xu, 2003). Mathematical tools have been

**Table 1**. Detection methods of pathogens in plants.

| Method | Advantage | Disadvantage |
|---|---|---|
| Visual Inspection (including teledetection) | Quick detection when the symptoms are well defined and clearly exposed. | Symptoms should be adjusted to one of the syndromes.<br>Soil hides symptoms.<br>Inspector should have ample experience. |
| Identification of pure cultures of pathogens | Most morphological taxonomic characters are well documented. | Production of pure *in vitro* crops is required and not quick or completely reliable.<br>Identification is not always easy if recent literature is not available.<br>Specified means of growth may not be available. |
| Biochemical methods | Substrate utilization has been well developed for bacteria of medical importance and biochemical methods have much potential to diagnose bacterial pathogens in plant pathology.<br>Chromatographic methods are now mature technology, including polyacrylamide-gel electrophoresis (PAGE). | Sufficient volume of an unknown isolate must be produced in pure culture for some chromatographic techniques including SDS-PAGE.<br>These methods are not very fast and have not been designed to be used easily in the field. |
| Microscopic exam | Viruses and bacteria can be examined by electronic microscope. | Requires a careful inspection by experts and equipment.<br>Electron microscopy requires expertise.<br>Microscopy is expensive. |
| Immunological methods | Most are simple techniques.<br>Most methods are quick.<br>Results are accurate and clear.<br>Pathogens which cause diseases with variable or latent symptoms on the host plant can be separated.<br>Pathogens with an indistinct structure or an undistinguished morphology such as in many groups of viruses and bacteria may be distinguished. | Specific methods have not yet been developed for most diseases.<br>Not effective for viroids which lack a protein coat. |
| Techniques for nucleic acids | Nucleic acid probes have already been prepared to a range of viral plant pathogens.<br>Hybridization tests are useful in quarantine for detect the unknown pathogens (including viroids). | Hybridization tests are not yet widely used against many fungi and bacteria.<br>Nucleic acid hybridization "dot blot' tests are likely to continue to be carried out only in a laboratory. |

employed to create models which provide a description of epidemic dynamics; the common mathematical tools used are: disease progress curves, linked differential equation (LDE), area under disease progress curve (AUDPC) and computer simulation. There are other tools that have been employed in epidemiology of plant disease like: statistical tools, visual evaluations and pictorial assessment (Contreras-Medina et al., 2009). The growth models commonly used are, monomolecular, exponential, logistic and gompertz. These describe the

disease' progress and can be represented by curves that indicate the severity with respect to time and distance (Contreras-Medina et al., 2009; Cooke et al., 2006; Madden, 1980; Nutter, 2007; Ojiambo et al., 2000). The evaluation is fundamental in the study and analysis of the disease epidemics in plants and is indispensable to estimate the crop loses and predict the expenses that will be incurred for its control.

Cooke et al. (2006) suggested evaluations using certain sampling methods such as; (a) random, (b) arbitrary (c) systematic and (d) stratified. The size of the sample is very important, that it is referred to in different methods like a graph, based on the coefficient of variability (randomized space, binominal negatives, binominal positives, Taylor´s relation) and the circle of possibilities.

## Quantification methods

Disease can be measured using direct methods or indirect methods. Direct methods are concerned with both the quantitative and qualitative estimations of disease (Cooke et al., 2006).

## Direct quantitative methods

In plant pathology, the three most common measures of disease are: (1) prevalence, (2) incidence, and (3) severity of disease (Cooke et al., 2006).

## Prevalence

Prevalence is defined as the number of geographical units (fields, farms, countries, states, regions, etc.) where a disease or pathogen has been detected, divided by the total of the geographical units evaluated (Cooke et al., 2006).

## Incidence

When measuring disease, one is interested in measuring the incidence of the disease. The incidence of the disease, is the number or proportion of plant units that are diseased (the number or proportion of plants, leaves, stems and fruit that show any symptoms) in relation to the total number of the units examined. This measure is continuously used in the epidemiological studies, to measure the propagation of a disease within a given field, region or country (Agrios, 2005). Incidence calculation is done through the following formula:

$$\text{Incidence (I)} = \frac{\text{Number of plants (or parts) x 100}}{\text{Total number of plants (o parts) observed}}$$

## Severity

When measuring disease, one is interested in measuring the severity of the disease, that is, the proportion of area or amount of plant tissue that is diseased (Agrios, 2005; Cooke et al., 2006). Measurement is said to be determined with greater accuracy according to the total seriousness of the disease, but such determination is slower and tend to be subjective when done solely by visual inspection, due to the variations or errors of visual acuity. Kwack et al. (2005) supported that visual assessment by the human eye usually tends to overestimate disease severity, especially with low levels of infection. Moreover, Lorenzini et al. (2000) suggested that the visual assessments are made quickly and do not require expensive equipment, chemical analysis or highly trained personnel, but their subjective nature creates concern and determines that the accuracy and precision of the measurement of injuries are questionable. Mirik et al. (2006) refers to the differences in assessments done by humans occur because individuals differ in their capability to perceive various wavelengths in visible spectra. The formula to calculate severity using an evaluation scale is:

$$\text{Severity (S) (\%)} = \frac{\text{(Number of plants x each degree) x 100}}{\text{Number of plants evaluated x highest degree}}$$

One of the recommended first steps in the study of epidemics new diseases is the development of reliable methods to estimate disease severity. Without reliable estimates, determination of disease progress rates, comparison of treatments such as cultivars or control measures and prediction of future disease or yield loss is not possible (O`Brien, 1992).

Disease assessment scales often are used for disease severity measurements. There are two general types of disease assessment scales: qualitative scales based on a subjective division of disease severity into levels and quantitative scales based on a quantitative trait, for example, percentage of the plant or plant part diseaded. Ideally, scales should be quick, easy to use, applicable over a range of conditions and reproducible, with sufficient intervals to represent all stages of disease development. Objective criteria such as accuracy, precision and correlation to yield loss should guide selection of one scale over another, but these criteria have rarely been used. Lack of standardization of disease severity scales may preclude comparison among experiments and/or observers (O`Brien, 1992). Table 2 shows the descriptiton, advantages and disadvantages of certain quantitative methods.

Visual methods are widely used due to their simplicity and cost. When using these methods, scales are most often used based on the fact that the majority to affect foliage or fruit is arbitrary selected as the interval between the different classes that is usually implemented to categorize different degrees of the disease´s intensity

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(Tovar-Soto et al., 2002).

Confection of evaluation scales of each disease in each crop will be carried out by the evaluator, depending on the pathogen´s characteristics, not only the symptoms are considered but also, the negative effect of the crop are measured. A number of studies have been reported elsewhere about using severity scales which includes; Anaya-López et al. (2003), Arzate et al.(2006), Chellappan et al.(2004), Dalla et al. (2005), Guigón and González-González, (2001), Hernández-Verdugo et al.

(2000), Holb et al. (2003), Kumar a et al. (2006), Latham and Jones, (2004), Maruthi et al. (2002), Mendez et al. (2002), Orlandini et al. (2008), Owor et al. (2004), Piper et al. (1996) and Torres-Limache (2004).

Relationships between incidence and severity are an epidemiologically significant concept; any quantifiable relationship between the two parameters may permit more precision. Three types of analysis have been used to describe this relationship, including, correlation and regression, multiple infection models and the measurement of aggregation (Cooke et al., 2006).

## Technological approaches in plant disease and pest detection

On the other hand, the use of technology has permitted to count utilizing computer programs that permit a comparison of the estimated severity with the real severity. Nutter and Schultz (1995) concluded that, the accuracy and precision of disease assessments was improved simply by selecting the most appropriate methods and by training observers to assess disease severity using computerized disease assessment training programs such as AREAGRAM, DISTRAIN and “Disease.Pro”. Although AREAGRAM graded user’s performance, it generated only standard area diagrams with fixed disease patterns. DISTRAIN was developed as a training programs for disease assessment using variegated patterns of disease severity for eight common foliar diseases of cereals; the program also allowed a comparison of estimated severity with actual severity. Nutter and Worawitlikit (1989) expanded the computer training concept in their advanced program for peanut diseases, “Disease.Pro” and later in 1998, developed a more generic disease assessment program, “Severity. Pro”, that allowed the user to select from a menu of leaf shapes (alfalfa, apple, barley, cucumber, grape, tomato) and lesion types (anthracnose, blotch, downy mildew, target spot, powdery mildew) so mimicking almost any foliar pathosystem (Cooke et al., 2006).

## Direct qualitative methods

Direct qualitative assessments of disease are used to differentiate host responses or interactions, ideally under controlled conditions, where resistance or susceptibility is determined by genetic systems in the host and pathogen. Qualitative assessment key have been developed to determine the resistance or susceptibility regarding the pathogen (Edwards et al., 1997; Khan and Boyd, 1969; Rosielle, 1972). In the field, the assessment of reaction types such as those described here is often more difficult than under controlled conditions, as host-pathogen interactions can be modified by environmental variables such as temperature and leaf surface wetness (Cooke et al., 2006).

## Indirect methods

Indirect methods of disease assessment have increased in number with the development of new technologies. Among these methods are remote sensor, infrared photography, image analysis, differential infrared thermometer, recount of spore and ELISA test (Maanen and Xu, 2003; Nutter et al., 2006; Nutter, 2007; Polishchuk et al., 2006; Vlugt, 2006). Thus, the chemical assays used for these biomarkers provide sophisticated quantitative techniques for the indirect assessment of disease severity in plant tissue. Other indirect methods use spore production as a measure of severity; Gough (1978) described a method for evaluating wheat cultivar response to *Septoria tritici* based on pycnidiospore production from soaked leaf segments using haemacytometer counts (Cooke et al., 2006).

Traditional methods, although still widely used, are rapidly being replaced by immunological and nucleic acid-based techniques. Of particular interest in the quantitative assessment of plant disease are user-friendly enzyme-linked immunosorbent assay (ELISA) kits for use in the field and the use of the polymerase chain reaction (PCR), particularly quantitative PCR (qPCR), for determining infection in plant material (Cooke et al., 2006).

## Remote sensing

The use of aerial photography and photogrammetry using infrared film or color filter combinations to enhance the differentiation between healthy and diseased tissue, represent a separate approach to disease assessment and were first used by Neblette (1927) and Taubenhaus et al. (1929) for surveying infection by cotton root rot (caused by *Phymatotrichum omnivorum*) in Texas and by Bawden (1933) in studies of virus diseases of potato and tobacco (Cooke et al., 2006).

Researchers currently have methods to measure the disease’s intensity using instruments with better visual accuracy. These methods analyze the differences between the damaged and healthy tissues. Some of these methods are based on the analysis of images and aerial photographs by means of infrared cameras that detect the differences of light reflection between healthy and diseased plants (Araus, 1998). These differences can be identified using thermal perception instruments, film photographs (natural or infrared color) or the analysis of digital image processes. This has created a big boom in remote perception that can be carried out through satellites and planes which permits monitoring of large crop areas in a short time and in a more practical way. Infrared film is usually used because near-infrared and infrared light are reflected deeper in leaf tissue than

visible light (Campbell and Madden, 1990). Early films were mainly analyzed using densitometry but, in later years, advanced image processing and spectral analysis were employed. Remote sensing now relies on digital image processing and image analysis, including advanced nuclear magnetic resonance imaging (NMRI), for the interpretation and quantification of non-destructive disease measurements in crops (Cooke et al., 2006).

Remote sensing for detecting and estimating severity of plant diseases is used at three altitudes or levels above the crop canopy. At the lowest altitude, within 1.5 to 2.0 m above crop height, hand-held multispectral radiometers or multiple waveband video cameras are used; at 75 to 1500 m, aerial photography is used, whereas at the highest altitude, satellite imagery is employed utilizing satellites orbiting at 650 to 850 km above the earth's surface (Cooke et al., 2006). An advantage of these methods is that the evaluation can be permanent. Unfortunately, the details are not clearly distinguishable because of the altitude at which the evaluation is carried out. In the same case the disease is hidden by healthy leaves when the levels of the disease are very low or the symptoms of the disease are found on basal leaves.

In 2002, image analysis software called ASSESS was made available by The American phytopathological society for plant disease quantification. The software was optimized for the measurement of leaf area, percentage of area infected, lesion/pustule count, root length and ground cover. ASSESS relies on the hue-saturation-intensity color model enabling the user to effectively extract the leaf from the background and then the lesions from the leaf (Cooke et. al., 2006).

The application of remote sensing to plant pathology lies mainly in the detection of crop the stress. A plant or plant population becomes stressed when a biotic or biotic factor adversely affects growth and development (Nilsson, 1995). Stress or disease can be expressed in various ways, such as imbalance in water supply leading to stomatal closure, decreased photosynthesis with associated changes in leaf fluorescence (Daley, 1995) and evapotranspiration and increased leaf surface temperature. Other symptoms may include leaf curling, wilting, stunting, chlorosis and necrosis of plant parts. Remote sensing provides a method for detecting and assessing such changes. However, it is likely that remote sensing will remain an indirect method of assessing plant disease through the interpretation of deviations from the norm, such as leaf temperature, rather than directly measuring reductions in leaf area due to disease (Cooke et. al., 2006).

Moreover, it has been reported that certain research in which color digital images have been used to identify pathogen agents, as demonstrated in the research of Camargo and Smith, (2009a), where artificial vision was presented for the identification of the visual symptoms of plant diseases. This began with color images. The digital images show unhealthy regions of the cultures that Camargo and Smith, (2009 b) referred to as being for the use of the segmentation of images in the detection of affected regions. Again, this method is not totally systematic because it depends on human observation. Solís et al. (2009) described an algorithm called LOSS which is capable of detecting white flies, some of which transmit disease. Recognizing and counting plague units within a plant nursery were carried out. This algorithm was designed considering geometrical characteristics of the white fly which include: (a) the projection area, (b) the eccentricity and (c) the solidity of the segmented insects. Kenneth (2009) presented the use of color infrared photography to detect disease of plants within greenhouse. Barón and Pineada (2009) detected early symptoms of diseases in plants using an instrument called "image fluorometer" which is capable of detecting diseases in plants and the movement of the pathogen within the plant; therefore, demonstrating its performance mechanism. This instrument is capable of diagnosing the "vegetal stress" (biotic like abiotic), caused by factors such as dryness, cold, heat and excess or lack of light. The image fluorometer captures images of red fluorescent which is dissipated by the pigment of the plants, diminishing its capacity to perform photosynthesis and receive excessive solar light. Traditional visual damage and disease quantifications in plants suffer from a lack of accuracy and precision. An alternative method that is consistent, unbiased and precise is computer automated digital image analysis. Computerized digital image analysis is also a non-destructive and non-invasive method that can capture, process and analyze information from images. Digital image analysis has been used in several studies to quantify disease (Mirik et al., 2006). There is still a lot of work to be done in this field of research to create new quantitative measures that are more accurate, precise and economical.

## QUANTIFICATION OF THE CHILI PEPPER VIRUS SYNDROME

Studies about quantification of chili pepper diseases, indicates that today visual assessment are used to estimate the severity of the disease in this crop, using severity scales, as shown in Table 3. These scales are composed of degrees, levels, categories, scales and/or values, of severity related to the symptoms observed. One of the most notable differences is the fact that some have more levels than others; furthermore, the established range or percentage is different and can be developed according to different necessities. There are no standards that permit comparisons between them, for example, when the degree and description of the symptom are different. Moreover, qualifying adjectives are used to describe the intensity and the magnitude of the disease's symptoms that again suggest subjectivity. Certain adjectives that are presented in such scale

**Table 3**. Scales of Severity used in quantification of pepper diseases.

| Used scale in the evaluation of the observed syndrome in regards to time in different types of infected peppers with the PHYVV and PepGMV | | Severity scale to evaluate the fungus and ash in chili pepper cultivation. Salaices, Chihuahua Mexico, 1998 | | | Severity Scale to evaluate damage by viruses in the chili pepper cultivation. Chihuahua, Mexico, 1998 | |
|---|---|---|---|---|---|---|
| Level description | | Category | Infected area (%) | | Category | Symptom |
| | | | Plant | Leaf | | |
| 1 | Mild wrinkling of the apicals leafs and the presence of light yellow dots of approximately 1mm diam. Only visible when exposing the leaves to light. | 1 | 0-25 | 0-30 | 1 | Yellow mosaic. |
| 2 | Appearance of light yellow dots in Isolated groups in the apicals leaves. | 2 | 26-50 | 0-30 | | |
| 3 | The groups of isolated dots begin to observe as a web mainly in the base of the apicals leafs. | 3 | 51-75 | 0-30 | 2 | Mosaic more shortening |
| 4 | The web is totally visible. | 4 | 76-100 | 0-30 | | |
| 5 | Protuberance formations shaped as *Insulas* in the middle of the leafs that at first manifest the symptoms. | 5 | 0-25 | 31-60 | 3 | Mosaic more shortening and more damage to the fruit. |
| 6 | The protuberances begin to lightly curl the leaf. | 6 | 26-50 | 31-60 | | |
| | | 7 | 51-75 | 31-60 | | |
| | | 8 | 76-100 | 31-60 | | |
| | | 9 | 0-25 | 61-100 | | |
| | | 10 | 26-50 | 61-100 | | |
| | | 11 | 51-75 | 61-100 | | |
| | | 12 | 76-100 | 61-100 | | |
| | Scale developed by: Torres (1997) | Scale used by: Guijon (2001) | | | Scale used by: Guijon (2001) | |

include; “mild” and “lightly” Torres (1997) scale; “light” and “lightly” (Hernández-Verdugo et al. 2000) scale; “few”, “more”, “some”, “severe”, “moderate”, “very” and “significant” (Pipper, 1996) scale. In this manner, neither the degree nor the description indicates with precision the intensity in which a symptom manifests itself, therefore, quantification may be questionable. In this sense, it is difficult to determine which scale is the best. Therefore, a short term alternative is to develop an accurate and precise standardized method of quantifying the syndromes of chili pepper disease that is used to predict the behavior of the diseases.

## FUTURE TRENDS

There is a notable trend toward more sophisticated solutions involving techniques of machine vision for detection and quantification of syndrome disease in plants. There is an increasing need for research that includes the development of tools to quantify the syndromes of the disease in chili crops, caused by geminiviruses such as *Pepper Huasteco yellow vein* virus (PHYVV) and *Pepper golden mosaic* virus (PepGMV). Also, the quantification must be standardized and used like a base for any syndrome virus-plant. Current analysis show subjectivity in the different scales; therefore, it is necessary to create quantification methods that consider the syndrome and respond to the following criteria: How mild, light, moderate, or severe is the intensity of the disease’s syndrome? One proposal of a quantifying method is shown in Figure 2 that includes a procedure to quantify the traditional form and a proposal of systematic quantification that outlines the use of processing digital colored images. With such devices, it is possible to obtain a less subjective quantification that generates accurate and precise data about the severity of the diseases and provide the necessary elements in order to make opportune choice, reducing losses in the production of the pepper crops. Obviously, the future will bring new technologies for detecting plant pathogens, largely because of the current efforts in genomics and molecular biosystematics and because of new platforms that have been developed primarily in the field of clinical medicine or even in the field of biological warfare. Whenever appropriate, they generally find their way somewhat later to plant pathogen diagnostics as well. Most progress can be expected from the development of simple and rapid devices for onsite pathogen detection. Recently, new formats using antibody-based detection for very rapid

**Table 3.** Continued.

| Scales of classifying leaf curl disease reactions caused by PepLCV in *Capsicum* | | Anthracnose severity scores and the symptom description on chili fruit and seedling leaf | | |
|---|---|---|---|---|
| **Severity grade** | **Symptom** | **Score** | **Chili fruit** | **Seedling leaf** |
| 0 | No symptom. | 0 | Infection absence. | - |
| 1 | 0 to 5% curling and clearing of upper leaves. | 1 | 1 to 2% of the fruit area shows necrotic lesion or a larger water soaked lesion surrounding the infection site. | Localized cellular death, lesions (<1 mm) with a defined margin – hypersensitive reaction. |
| 2 | 6 to 25 curling, clearing of leaves and swelling of veins. | 3 | >2.5% of the fruit area shows necrotic lesion, acervuli may be present/or water soaked lesion up to 5% of fruit surface. | Small isolated necrotic lesions covering approximately 1% of the leaf area. |
| 3 | 26 to 50% curling puckering and yellowing of leaves and swelling of veins. | 5 | >5 to 15% of the fruit area shows necrotic lesion, acervuli present/or water soaked lesion up to 25% of the fruit surface. | Larger discrete necrotic lesions covering approximately 5% of the leaf area. |
| 4 | 51 to 75% leaf curling and stunted plant growth and blistering of internodes. | 7 | >15 to 25% of the fruit area shows necrotic lesion with acervuli. | Coalesced necrotic lesions covering approximately 10% of the leaf area, acervuli presence. |
| 5 | More than 75% curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set. | 9 | >25% of the fruit area shows necrotic, lesion often encircling the fruit, abundant acervuli. | Coalesced necrotic lesions covering > 25% of the leaf area with abundant acervuli. |
| Scale developed by Banerjee (1987) and used by:Kumar et al. (2006) | | Scale developed by:Montri et al. (2009 and used by Cai et al. (2009), and modified by Mahasuk et al. (2008) | | |

**Table 3.** Continued.

| Severity scale used on bell peppers | | Severity scales of the disease on species *C. annuum* | |
|---|---|---|---|
| **Grade** | **Description** | **Severity grade** | **Symptom** |
| 0 | No visual disease symptom. | 0 | Symptom absence. |
| 1 | Vascular discoloration or stem necrosis. | 1 | Light distortion of the apical leafs and yellow dots on the leaves exposed to sunlight. |
| 2 | Vascular discoloration and stem necrosis. | 2 | Visible yellow dots on isolated apical zones of the leaves. |
| 3 | Wilting and no vascular discoloration. | 3 | Isolated yellow dots begin to unite forming a web on the base of the apical leaves. |
| 4 | Wilting and vascular discoloration. | 4 | Visible webs of yellow stains clearly configured. |
| 5 | Death. | 5 | Wrinkles in the middle of leaves. |
| | | 6 | Light curving of the leaves. |
| | | 7 | Light leaf distortion wrinkling of the leaves. |
| | | 8 | Distortion of the entire leaf. |
| | | 9 | The leaves of the infected plants, less than the controlled plants. |
| Scale used by: Sanogo (2006). | | Scale proposed by: William (1988) and used by Hernández-Verdugo et al. (2000). | |

presumptive on-site diagnosis have become available. These do not require specialized equipment or knowledge.

## CONCLUSION

There are few methods of quantification to evaluate the

**Table 3.** Continued.

| Scale used to evaluate the disease at the root, the leaf and fruit | | | | | | Scale used for rating severity of SCMV-MDMV-B and MDMV infection on eastern gamagrass | |
|---|---|---|---|---|---|---|---|
| Root | | Leaf | | Fruit | | Value | Symptom description |
| Scale 'A' | Tissue with disease (%) | Scale 'B' | Tissue with disease (%) | Scale 'C' | Diameter of the lesion (mm) | | |
| | | | | | | 0 | Healthy, no virus visible. |
| 1 | < 2 | 1 | < 2.5 | | < 2 | 1 | Very mild symptoms on one or more leaves. Mosaic not extremely distinct and little yellowed area on any symptomatic leaf. |
| 2 | 2.1-10 | 2 | 2.6-7.5 | 1 | 2.1-4 | | |
| 3 | 10.1-25 | 3 | 7.6-12.5 | 2 | 4.1-6 | | |
| 4 | 25.1-45 | 4 | 12.6-17.5 | 3 | 6.1-10 | 2 | Mild symptoms on one or more leaves. Symptoms more distinct and /or more leaf area with mosaic than in 1. Not necessary more leaves infected. |
| 5 | 45.1-75 | 5 | 17.6-32.5 | 4 | > 0 | | |
| 6 | > 75 | 6 | 32.6-67.5 | 5 | | | |
| | | | | | | 3 | Moderate symptoms on one or more leaves. Mosaic distinct, even bright. More leaf area, and/or more leave with symptoms than 1 or 2. |
| | | | | | | 4 | Moderate symptoms (as in 2 or 3) but more widespread than 3. Some healthy tillers present. Especially, more symptomatic leaves per tiller. No necrosis or stunting visible. |
| | | | | | | 5 | Severe symptoms, widespread on plant. Especially, most to all leaves on a tiller showing symptoms. |
| | | | | | | 6 | Severe symptoms, as in 5, but in addition either noticeable stunting or small to moderate amount of necrosis. |
| | | | | | | 7 | Very severe symptoms, severe stunting, obvious and significant amount of necrosis. |
| Scale used by Holb et al. (2003) | | | | | | Scale used by Pipper (1996) | |

syndrome in chili plants caused by viruses available, more specifically for the chili pepper. The quantifications currently reported, suggest ambiguity within the evaluation scales used to measure symptoms severity. These methods do not indicate the degree when a symptom is present, such as is in the case of the scale developed by Torres (1997). A specific example is when an evaluator from a scale, assigns a value to determine that the plant shows wrinkling, this value does not indicate in real terms, the magnitude of wrinkling symptom. Thus, these situations suggest that further discussion is necessary. Furthermore, the fact that evaluators generally carry out quantifications for severity through visual observations and the criteria from one evaluator to another is different and can be problematic. Quantification disease's syndromes are still not very addressed or diffused in the currently published research. Finally, in scales, a disadvantage is the different degrees and categorization from severity besides using numbers, percentage, adjectives denominations and description of the disease. There is not even an agreement to evaluate different parts of the plant. Scales are developed by different people with particular interests; there are not associations that define standards to quantify plants. One of the most important disadvantages is that the quantification of plant diseases on the world-wide level is backward in progress; the quantification is a delicate situation where furthermore, the syndrome and the interpretation differed from one another. Therefore, there needs to be fewer suggestive systematic methods that improve productivity of crops and that permit early detection of plant disease symptoms for chili pepper crops, for example, by means of analyzing color images, identifying in an early way, plant disease symptoms in chili crops with the objective of providing producers with a tool that will better predict the behavior of the diseases caused by the viruses. For this reason, the search for biotechnological alternatives that may increase the productivity of this Solanaceae member is necessary.

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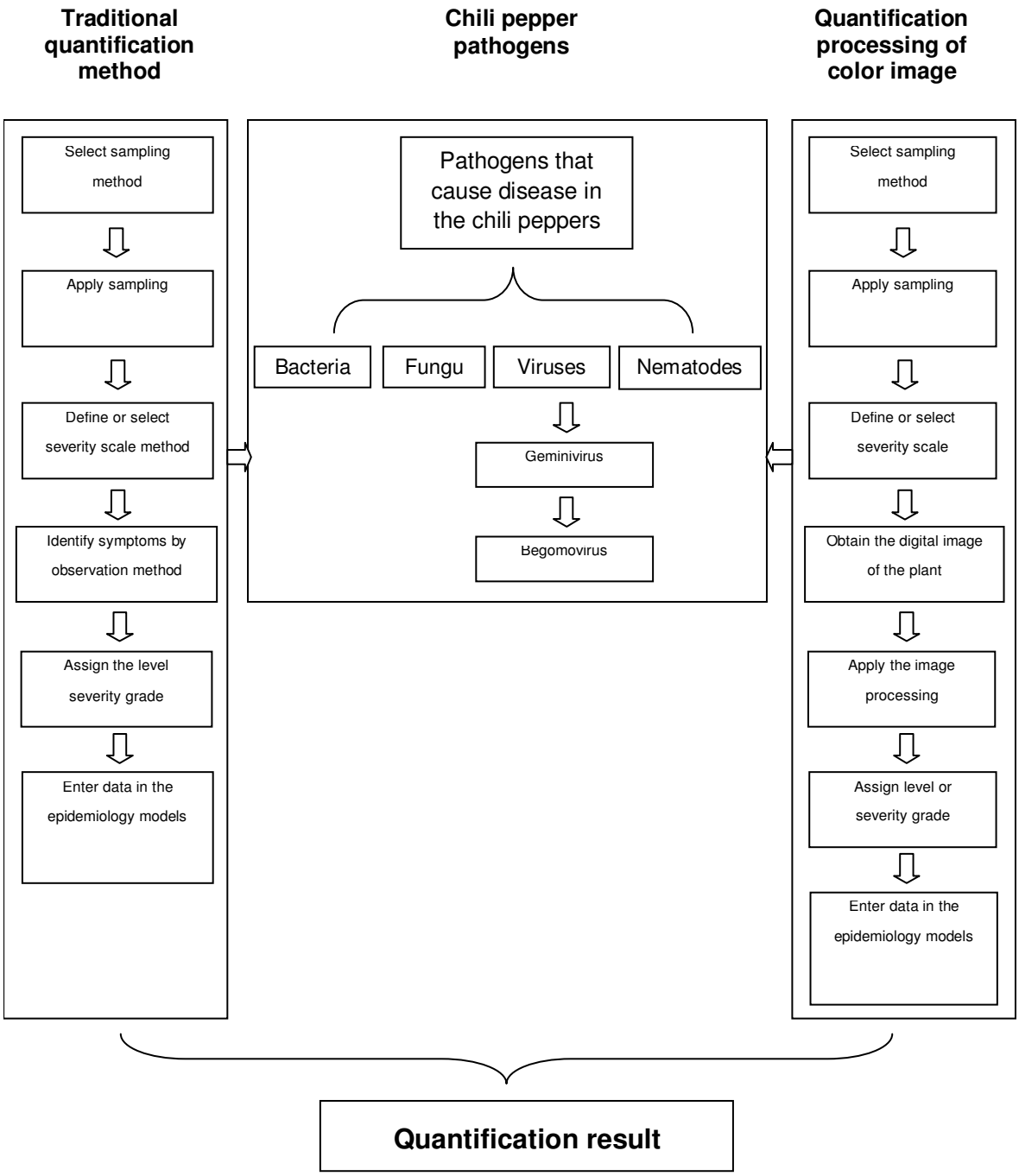


**Figure 2.** Quantification scheme for the chili pepper's disease syndromes for traditional methods and proposal of systematic quantification using digitally colored image processing.

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