

5 Estimating Disease Severity and Incidence

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5.1 Role of Disease Assessment in Root Disease Epidemiology

Disease assessment is one of the most challenging tasks in working with plant diseases. A diseased plant or group of diseased plants is often recognized easily once symptoms or signs become visible; however, it is the quantification of the disease that presents the challenge. The assessment of disease incidence (i.e., the number or proportion of diseased plants in a population) is an apparently simple counting task, but is subject to the usual limitations of interpretation related to sample size. The accurate and precise estimation or measurement of disease severity (i.e., the area or proportion of plant tissue that is symptomatic) can be a formidable task because of visual and measurement errors and the need for samples to be representative of the area considered and to be of adequate number.

Many factors influence the quality of disease assessment data. Our objectives in this chapter are to examine these factors through (1) the identification of the needs and goals for disease assessment; (2) consideration of the types of symptoms and signs that may be assessed along with the challenges associated with such assessments; and (3) analysis of the methods and techniques available to quantify root diseases. We will also address some of the challenges in disease assessment that are unique to root disease epidemiology.

5.1.1 The Need for Disease Assessment

Disease assessment, the cornerstone of epidemic analysis, is the most important task in the study of plant disease epidemics. It is the starting point in the characterization of any epidemic and represents the foundation for all subsequent analyses and interpretations. In essence, assessments of disease intensity are the currency by which epidemics are characterized and compared.

Assessments of disease are vital to our interpretation as to whether disease management practices are successful. Relative magnitudes of success in disease management are judged on a comparative basis by growers and

scientists through the process of disease assessment. The ultimate extension of such assessments is the translation of impressions of how much disease is present into mental or mathematical estimates of crop losses due to disease.

In many cases, assessment of disease during the course of root epidemics is the most costly part of a study. The cost is apportioned among the time needed to plan and carry out the assessment, the equipment required, and the degree of crop destruction brought about by the assessment. Quite often, the time component provides the largest real cost factor in an assessment of root disease intensity.

5.1.2 Goals of Disease Assessment

The goals of an assessment depend on a clear conception and statement of the objectives of the particular study. A survey of fields of crop x in region y to determine the prevalence of root disease z will have different assessment goals than a study to determine how moisture stress and soil physical factors affect the spatiotemporal progress of disease z on crop x in one or two fields. Not only will the goals differ, but the data quality objectives will undoubtedly be different for two so different studies. Thus, the specific goals for disease assessment must be formulated for each specific study.

The general goal of disease assessment, however, will remain constant for all types of studies. This goal is to provide *reliable* estimates of the *amount of disease* in an *area* (plot, field, farm, county, region, etc.) based upon the evaluation of specific symptoms and signs, which are known to be *characteristic for a disease*, at the lowest reasonable *cost* with *known confidence*. Few actual disease assessments fulfill this goal completely. An examination of the key components of this general goal is instructive in developing and evaluating disease assessment schemes.

Disease assessment must be *reliable*. The estimates or measurements of disease should be accurate, precise, and reproducible. *Accuracy* refers to the closeness of the sample mean from the assessment to the true population mean and is measured as bias, where $\text{bias} = |\bar{x} - u|$ with \bar{x} = the sample mean and u = the population mean. Bias is difficult to measure in most actual field studies, because the required knowledge of the population mean is usually not available. Bias can, however, be measured in a preliminary study where a census of plants is taken in an area and measurements of the amount of disease are made. A sample mean can then be obtained by assessing disease in the manner that would be used under actual field conditions on a set of plants chosen randomly. The true mean and the sample mean can then be compared to estimate the bias.

Precision is a measure of the relative closeness or scatter of estimates obtained about the mean and is estimated by sample variance, s^2 . The greater the sample variance, the lower the degree of precision in estimating \bar{x} . A low degree of precision (= high s^2) can be obtained due to errors in the

assessment or because of actual variation in the relative amounts of disease on individual plants. The degree of spatial aggregation will have a significant influence on the magnitude of s^2 obtained for root diseases (see Chap. 7).

Reproducibility, a measure of the ability of an evaluator to obtain the same estimate of disease at a second, third, fourth, etc. assessment of the same diseased plant, plot, or other area within a short time interval, can be estimated by the degree of correlation between values from sets of assessments for the same plants, plots, etc. made at different times within a short time interval by the same evaluator. If the values for the amount of disease in populations of specific plants or plots from two assessment times are compared by a correlation analysis, a high degree of correlation (i.e., a coefficient $r \geq 0.80$) suggests a high degree of reproducibility for the assessment.

A slightly different approach to measuring reliability was taken by Shokes et al. (1987) in the assessment of the foliar disease of late leaf spot of peanut; however, the approach should be equally applicable to root diseases. The goal was to provide a measure of agreement among several disease evaluators or judges (i.e., inter-rater reliability) to indicate how reliable estimates of disease were. Each judge estimated the amount of disease present in each of several plots and variance components associated with the true variation among plots (σ_T^2), the variation among evaluators (σ_j^2), and the interaction among evaluators and plots or error (σ_c^2) were calculated. Variance components were used to calculate a coefficient of reliability (ρ) such that

$$\rho = \sigma_T^2 / (\sigma_T^2 + \sigma_j^2 + \sigma_c^2).$$

The greater the magnitude of ρ , the greater the degree of inter-rater reliability. Such a measure will be quite useful if it is necessary to have more than one disease evaluator, particularly during evaluator training.

The way in which the *amount of disease* is estimated or measured will depend on the type of disease and purpose of the study. *Disease intensity* refers to the quantity of disease present in an area (Seem 1984; Kranz 1988; Campbell and Madden 1990). Within disease intensity, the measures of incidence and disease severity can be distinguished. *Disease incidence* refers to the number of plant units (e.g., plants, primary roots, secondary roots) that are visibly diseased (James 1974; Kranz 1988; Campbell and Madden 1990). *Disease severity* is the area or volume of plant tissue that is visibly diseased (Kranz 1988; Campbell and Madden 1990) relative to the total area or volume. *Disease prevalence* can be used as an extension of disease incidence when it is taken to be the percentage or proportion of fields in a region in which the disease is observed (Zadoks and Schein 1979).

Area is an important component of disease assessment because it indicates the spatial or geographic scale at which the assessment was made. Whereas it may be possible to examine the co-occurrence of events at a regional scale, at the scale of a rhizosphere, it is possible to examine direct

causes, e.g., different symptoms due to different pathogens. The complexity of the logistics associated with the assessment of disease, as well as the relative accuracy and precision of the values obtained, will vary as the spatial scale increases from that of a single plant or rhizosphere to a county or region. Disease assessment at a regional scale will involve more evaluators and a longer period of time will be required for an assessment. Changes that occur during the assessment period will be more difficult to take into account for a regional assessment than those that occur in a relatively shorter assessment period for a single field. Techniques available to estimate or measure disease intensity and the general sampling approach will also vary with the scale of interest. Because of differences in the relative precision of the techniques used, estimates of disease from different spatial scales may not be comparable directly. Expansion factors for data obtained in small areas to provide estimates of disease for larger areas (e.g., regions) should be considered prior to developing the sampling plan.

With root diseases there are often several types of symptoms and signs associated with a single disease. There may be both root and shoot symptoms (see Sect. 5.3) that are *characteristic for a disease*. For example, a plant with root or crown rot caused by a *Phytophthora* spp. may first exhibit lesions on small roots or near the crown; however, the first visible (i.e., aboveground) symptom may be wilting of the shoots and leaves. Care must be taken to insure that the wilting is indeed due to the root disease and not purely to drought stress or plant injury. Stunting of plants is another symptom that may be characteristic of a root disease, but could also be due to other causes.

Cost is a factor of primary importance in planning the assessment of disease. Cost of effort must be balanced against the need for a certain amount of information. In experimental plots associated with specific research projects, the expenditure of large amounts of time and effort may be warranted to fulfill the objectives of the study. The intensive efforts required make the estimates of disease intensity high-priced, but such cost is expected with research. In survey assessments, however, the need may be more to cover a given area within a specified amount of time. In this case, as indeed in all cases, the cost of the estimates must be balanced against the accuracy and precision of the estimates obtained. More accurate and precise estimates are usually more costly than estimates with a lower accuracy and precision.

Data quality objectives and statistical confidence are issues that require much more attention in disease assessment than they have received previously. Both are attributes of the *known confidence* that must be associated with assessments of disease. A data quality objective is a statement of the magnitude of change that must be detectable for a disease over a specific time interval. Certain levels of statistical confidence are required for the establishment of the data quality objective. For example, based upon a knowledge of yield losses or a set of management decision rules, an investigator may decide that a 5% change in the level of disease is the smallest

interval to be detected. The time interval of interest is a 2-week period. Thus, the first part of the data quality objective would be: *to detect a 5% change in disease within a 2-week period*. Statistical confidence in reaching the data quality objective comes from assigning specific, acceptable levels of α (probability of making a type I error, i.e., falsely rejecting the null hypothesis or identifying differences that actually do not exist) and β (probability of making a type II error, i.e., falsely accepting the null hypothesis or failing to identify differences that actually do exist). It may be reasonable to set α at 0.05, so that the probability of not making a type I error is 0.95 and to set β at 0.10 so that the probability of not making a type II error is 0.90.

The issue of degree of statistical confidence in the data will have an impact on assessment costs and is important in designing the assessment scheme. To gain greater confidence in assessment data, evaluators' errors can be reduced and sample size (n) can be increased. Also, if the level of differences to be detected is, for example, 2%, the use of a disease assessment or rating scale with a resolution of only 5% is not appropriate. Similarly, a five-point scale (0 = healthy, 1–25, 26–50, 51–75, 76–100%) would not be appropriate to detect a 2% change. Thus, data quality objectives need to be considered in relation to the overall purpose of the assessment and the resources available to obtain the assessment data needed.

5.2 Signs and Symptoms

Signs are the parts of a pathogen or its products seen on the diseased host plant. *Symptoms* are the internal or external reactions or alterations of a plant due to disease (Lucas et al. 1992). Most assessments of root diseases rely on root and shoot symptoms; however, in those instances where signs are present and readily observed, they can serve as confirmatory evidence of the presence of a specific pathogen. Signs associated with root diseases most often include sclerotia of fungi such as *Sclerotium rolfsii* and *Sclerotinia* spp., the mycelium of certain fungi such as *Rhizoctonia solani*, or basidiocarps of certain wood-decaying fungi such as *Armillaria mellea*. For root diseases caused by nematodes such as *Heterodera* spp., the presence of visible cysts is a characteristic sign.

Symptoms associated with root diseases range from minute lesions on fine rootlets to wilting of the entire plant. The dilemma in assessment of root diseases is that the symptoms on roots will often precede symptoms visible on aboveground portions of plants, but shoot symptoms may be much easier to evaluate than root symptoms. This dilemma is discussed further in Section 5.3. The point here is to raise the issue of what symptoms are to be evaluated to fulfill the general goals of assessment identified previously.

5.3 Root and Shoot Symptoms

Concepts applied to assessment of foliar diseases can be utilized for root diseases (Kranz 1988). However, symptom assessment of root diseases present a challenge not equaled by foliar diseases. Direct assessment of roots requires destructive sampling which prevents repeated assessment on individual roots or plants through time and may influence epidemic development in plant populations (Table 5.1). The "picture" of the epidemic may also change because variation over time (= different sample units at each time) may not be at all constant, especially with diseases characterized by aggregation and/or clustering of diseased plants. If the assessment is conducted only at harvest, roots may have been sloughed prior to sampling and, thus, an underestimate of disease intensity may be obtained (Berger 1980).

It is tempting to rate only the shoot symptoms associated with many root diseases because of the problems associated with actual root assessments. However, shoot symptoms may differ quantitatively and qualitatively

Table 5.1. Advantages and disadvantages of destructive and nondestructive sampling for the assessment of root diseases

Sample type	Advantage	Disadvantage
Destructive	<ul style="list-style-type: none"> - Actual root evaluated, not a surrogate - Allows determination of causal agent - Assessment of reduction in root volume is possible when compared to healthy plants - Sampling units independent or nearly so 	<ul style="list-style-type: none"> - Sample unit is destroyed - Adjacent plant roots and root environment are disturbed - Usually very time-consuming and costly - Disease progress cannot be observed temporally on same plants
Nondestructive	<ul style="list-style-type: none"> - Plants remain intact and can be evaluated repeatedly - Root environment remains undisturbed - Sample units independent only if new sample of plants evaluated each time 	<ul style="list-style-type: none"> - Visible, above ground symptoms may have a range of temporal and physiological relationships to root symptoms - Assessment values may be autocorrelated if the same plants are evaluated repeatedly - No estimate of reduction of size in root system is obtained - No estimate of severity of symptoms on roots

from root symptoms and often develop at variable times after root infections occur. Physiological, environmental, and temporal relationships between development of root and shoot symptoms should be understood before a specific symptom type is selected for monitoring epidemics and evaluating management procedures.

5.3.1 Physiological Relationships

In some cases, the actual effects of root disease are greater than the visual estimates of damage to roots would indicate. For example, when root diseases cause plants to wilt in a relatively wet soil under mild transpirational stress, large resistances to water uptake by the plant may have developed. At all stages of the development of *Phytophthora* root rot in safflower, resistance to water flow through healthy and infected safflower roots was much greater than would be expected due to differences in the size of root systems (Duniway 1977). Because the reduction in root surface area only accounted for a portion of the resistance to water flow induced by infection, a large portion of that resistance to flow in infected roots could be attributed to the xylem (Duniway 1977). In another system, *Cylindrocladium* black rot of peanut, stem and leaf wilting may result from reduced water flow caused by an accumulation of gums in vessels of fibrous and tap roots (Harris and Beute 1981).

In order to ascertain the physiological effects of disease on plants, the position and depth of lesions on roots must be evaluated (Hornby and Fitt 1981). Fitter (1982, 1987) defined a quantitative morphometric root analysis system which classified root systems by root order (first, second, or third) and type (lateral, tap root) (Fig. 5.1). The type and order of root segment may influence the susceptibility of root tissue as shown for infections of tobacco roots by *Phytophthora parasitica* var. *nicotianae* (English and Mitchell 1989). Also, infections and/or lesions on third-order (tap) roots would likely reduce water flow to stems more than lesions on first- or second-order (lateral) roots (Fig. 5.1; Bowers et al. 1991). This was apparent for *Phytophthora* root rot on processing tomato (Neher 1990) and tobacco black shank (Jones 1990) where symptoms on shoots were visible when the tap root had lesions, but not when lesions were restricted to lateral roots.

Some root diseases may elevate or alter levels of plant hormones that at least partially account for symptoms visible on roots or shoots. For example, increased ethylene production occurs with *Verticillium* wilt and plays an interactive role in the chlorosis and wilting of tomato leaves, as well as in the defoliation of cotton plants (Pegg 1981). Plant hormone shifts can alter patterns of photosynthate partitioning as illustrated by corky root of lettuce (O'Brien and van Bruggen 1992b) caused by *Rhizomonas suberifaciens* (van Bruggen et al. 1990). Diseased roots were significantly greater in diameter, had greater biomass, and much lower water content than apparently healthy

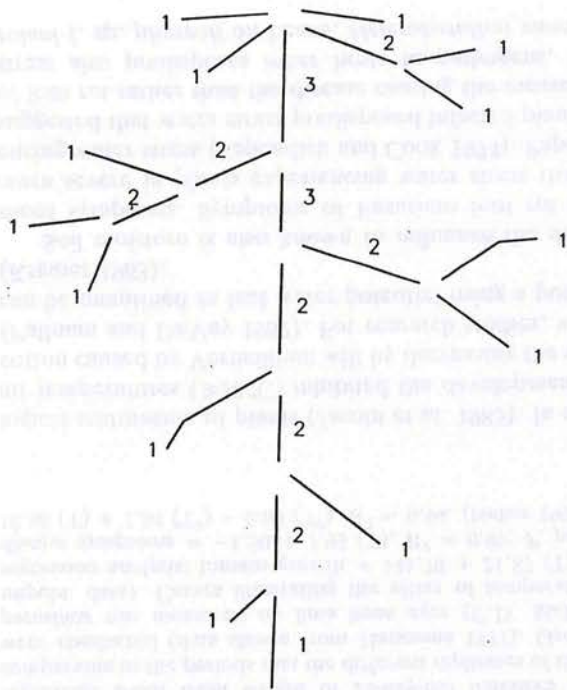


Fig. 5.1. Schematic representation of a pepper root system broken into root segments and described by the classification scheme of the morphometric root analysis system; 1, 2, 3 = root orders. (Bowers et al. 1991)

roots; more photosynthate was partitioned to roots relative to shoots, thus resulting in "corkiness" of the tap root (O'Brien and van Bruggen 1992b).

Mineral deficiencies and toxicities can confuse the interpretation of disease symptoms. Mineral nutrition may accentuate disease symptoms (Huber and Watson 1974) or lead to an incorrect diagnosis of the cause of a plant's poor health, especially by an inexperienced observer. For example, nitrogen-deficient hop plants may not show symptoms even though *Verticillium* spp. can be isolated from roots (Huber and Watson 1974). The type and abundance of nutrients in soil can also influence the severity of disease. For example, different forms of nitrogen, i.e., nitrate or ammonium, may differentially increase or decrease the severity of disease symptoms. No single form of nitrogen influences symptom development the same way for all diseases; therefore, each disease must be considered individually. For example, cortical and root diseases caused by species of *Fusarium*, *Rhizoctonia*, *Aphanomyces*, *Cercospora*, *Poria*, and *Armillaria* may be reduced by applications of $\text{NO}_3\text{-N}$ and increased by applications of $\text{NH}_4\text{-N}$, whereas diseases caused by species of *Ophiobolus*, *Diplodia*, *Pythium*, and *Strept-*

omyces respond in an opposite manner (Huber and Watson 1974). The nitrogen form can affect the composition of root exudates which in turn may influence pathogenesis (Schroth and Hildebrand 1964; Huber and Watson 1974). In addition, nitrogen may influence microbial interactions, for example, suppression of pea root rot in soils amended with $\text{NO}_3\text{-N}$ was correlated with an increased abundance of bacteria and actinomycetes antagonistic to the pathogen, *Aphanomyces euteiches*; the relationship was not observed in soils amended with $\text{NH}_4\text{-N}$ (Carley 1969).

5.3.2 Environmental Effects

Temperature, moisture, and salinity stresses may influence the expression of root and shoot symptoms, as well as the activity of other root-infecting organisms. The effects of temperature may differ for each the pathogen, host, and disease. The most severe symptoms on processing tomato roots caused by *Phytophthora parasitica* occurred at temperatures that surpassed those favorable for either tomato growth or *P. parasitica* alone (Neher 1990; Fig. 5.2). A different relationship was observed, however, for *Phytophthora* root rot on avocado. The growth curve of *Phytophthora cinnamomi* in response to temperature was similar to that of avocado, except at 33°C where the host grew well, but the pathogen did not (Zentmyer 1981). Disease development was greatest at $15\text{--}27^\circ\text{C}$, but the host outgrew the pathogen at 33°C (Zentmyer 1981). In the case of black root rot of tobacco, the temperatures optimum for the pathogen, *Thielaviopsis basicola* (= *Chalara elegans*), and its tobacco host were similar ($28\text{--}30^\circ\text{C}$) (Johnson and Hartman 1919, cited by Zentmyer 1981). However, the disease symptoms caused *T. basicola* on tobacco were most severe at $17\text{--}23^\circ\text{C}$, and little damage occurred at $28\text{--}30^\circ\text{C}$ (Zentmyer 1981). Apparently, *T. basicola* was unable to cause disease in a vigorously growing plant at the higher temperatures. In contrast, disease development in corn and wheat, infected with *Gibberella* spp., was most severe at temperatures unfavorable for the host (Dickson 1923, cited by Zentmyer 1981).

The development of wilt symptoms may be influenced by high transpirational demand as in *Phytophthora* root rot in processing tomato, caused by *P. parasitica* (Neher 1990) and black shank of tobacco, caused by *P. parasitica* var. *nicotianae* (Jacobi et al. 1983). For processing tomato, soil temperatures influenced the severity of root symptoms that developed before shoot symptoms were apparent (Neher 1990; Fig. 5.3). Infections by *P. parasitica* on tomato and parsley and *P. cinnamomi* on avocado occurred at low soil temperatures, but symptoms were delayed until soil temperatures exceeded a threshold level during an early vegetative phase of the host (Hine and Aragaki 1963; Zentmyer 1981; Neher 1990). Black shank symptoms developed rapidly on tobacco after periods of drought and high transpiration. These stressful environmental conditions coincided with physio-

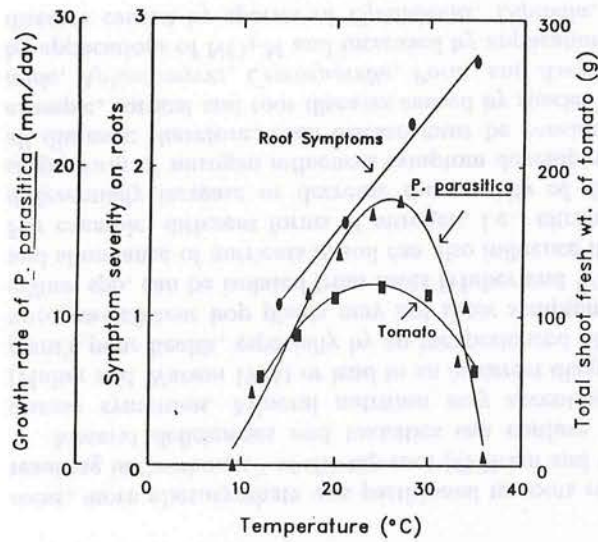


Fig. 5.2. Effect of soil temperature on disease development (ovals) and tomato growth (squares) and substrate temperature on in vitro vegetative growth of *Phytophthora parasitica* (triangles). Disease severity values represent the mean root symptom severity across replicate experiments of a greenhouse study. Tomato growth represents shoot fresh weight of 28-day-old tomatoes averaged for the seasons comparable to the periods that the different replicates of the greenhouse experiments were conducted (data shown from Harssema 1977). Growth rate (mm/day) of *P. parasitica* was measured on lima bean agar (C.D. McKeen and J.M. Duniway, unpubl. data). Curves illustrating the effect of temperature (T) were fitted using regression analysis: tomato growth = $141.70 + 21.87(T) - 0.45(T^2)$, $R^2 = 0.96$; disease symptoms = $-1.50 + 7.93(T)$, $R^2 = 0.99$; *P. parasitica* growth = $2.38 - 10.86(T) + 1.64(T^2) - 0.04(T^3)$, $R^2 = 0.94$. (Neher 1990)

logical maturation of plants (Jacobi et al. 1983). In contrast, periods of high air temperatures ($\geq 28^\circ\text{C}$) inhibited the development of foliar symptoms of cotton caused by *Verticillium* wilt by decreasing the susceptibility of the host (Pullman and DeVay 1982). For research studies, wilt symptoms on shoots can be quantified as leaf water potential using a portable pressure chamber (Kramer 1983).

Soil moisture is also known to influence the development of root and shoot symptoms. Symptoms of *Fusarium* foot rot on mature wheat were more severe in plants experiencing water stress than in plants not experiencing water stress (Papendick and Cook 1974). Papendick and Cook (1974) suggested that water stress predisposed infected plants to rapid development of foot rot rather than the disease causing the measured water stress. Water stress also predisposes other hosts to pathogens, for example, *Fusarium solani* f. sp. *phaseoli* on beans, *Heterobasidion annosum* on pine seedlings,

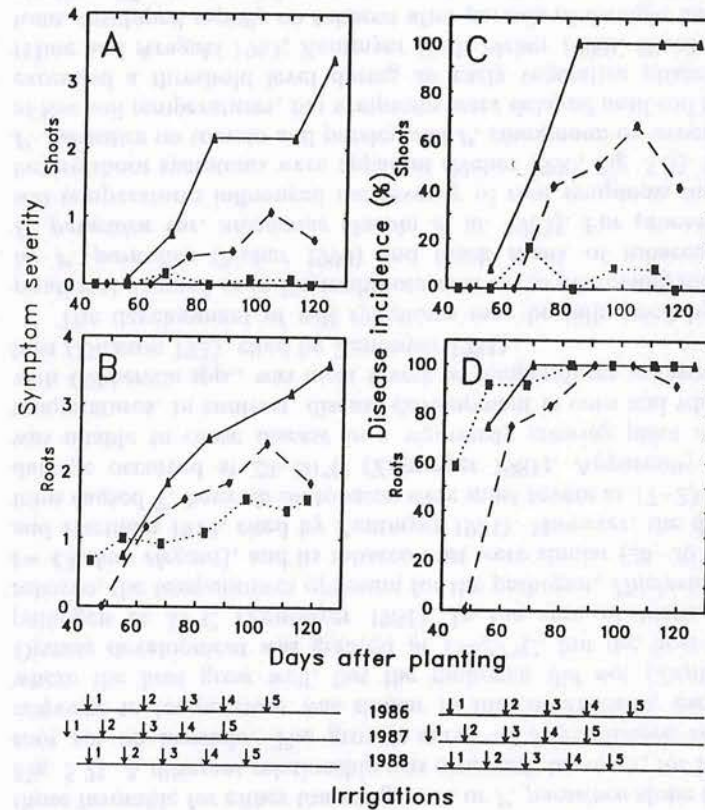


Fig. 5.3A-D. Mean severities of symptoms on shoots (A) or roots (B) and the incidence of processing tomato plants with shoot (C) or root (D) symptoms of *Phytophthora* root rot plotted as a function of days after planting in 1986 (triangles) (data for 1986 from Ristaino et al. 1989), 1987 (squares), and 1988 (diamonds). Symptom severity was based on a scale of 0-4 where 0 = healthy and 4 = extreme symptoms. Values represent means of 12 plants destructively sampled in four replicate plots. Arrows represent the dates of irrigations. The first irrigation was always 2 days after inoculation. (Neher 1990)

and *Macrophomina phaseolina* on sorghum and cotton (Cook and Papendick 1972). In contrast, wet soils favor development of *Phytophthora* root rots by creating environmental conditions favorable to growth and dispersal of the pathogen (Duniway and Gordon 1986).

If some factor of the environment is unfavorable to the host, various physiological processes, including disease resistance, are commonly impaired (Cook and Papendick 1972). For example, flooding, drought, and salinity stresses may predispose various hosts to *Phytophthora* root rot (Duniway

and Gordon 1986). The mechanisms of predisposition are not known clearly but low oxygen levels in saturated soils may make roots leak unusually large quantities of root exudates and, thus become more attractive to zoospores (Kuan and Erwin 1980). In addition, a lack of regeneration or growth of new roots under drought or saline conditions in soil may impair a plant's capacity to tolerate root rot (Duniway and Gordon 1986).

Plants can be predisposed to root diseases caused by fungi and bacteria by other root-infecting organisms, such as nematodes. Root cracking, galling, and giant cell formation caused by *Meloidogyne incognita* may predispose cotton to other root diseases, e.g., Fusarium wilt (Shepherd and Huck 1989). Nematodes may also interact synergistically with other disease organisms to increase the relative incidence and severity of symptoms (Powell 1971; Shepherd and Huck 1989). Inoculation of tobacco with *Pratylenchus brachyurus* simultaneously or 1 week before *Phytophthora parasitica* var. *nicotianae* increased the severity of black shank symptoms in comparison to those observed when *P. parasitica* var. *nicotianae* was added alone (Inagaki and Powell 1969). This suggests that black shank symptoms developed faster in plants that were wounded mechanically by the nematodes. Peanuts were predisposed to *Cylindrocladium* black rot mostly by the nematodes *Meloidogyne hapla* and less by *Macroposthonia ornata* (Diomande and Beute 1981). Severity of foliar symptoms and yield loss were greater for potatoes with early dying disease when *Verticillium dahliae* was present in combination with *Pratylenchus penetrans* than when *V. dahliae* or *P. penetrans* was present alone (MacGuidwin and Rouse 1990).

5.3.3 Temporal Aspects

A time lag often occurs between infection by the pathogen and symptom development in roots, and between symptom expression on roots and shoots. For example, colonization of cotton roots by *Verticillium dahliae* lagged increases in inoculum density by 3 to 4 weeks (Huisman 1988). Infection by *Phytophthora parasitica* var. *nicotianae* may occur on tobacco roots soon after transplanting, but shoot symptoms of black shank may not be apparent until 5–6 weeks after infection (Jacobi et al. 1983). Visible symptoms of *Phytophthora* root rot were not apparent on tomato roots until approximately 9 days after infestation of soil under field conditions; shoot symptoms were not apparent until at least 14 days after root symptoms were apparent (Neher and Duniway 1991).

The expression of symptoms may be linked to host plant phenology. The expression of symptoms of *Phymatotrichum* root rot on cotton is closely associated with cortical senescence in roots. Disease symptoms are restricted initially to the roots, and foliar symptoms are not visible until the root cortex is sloughed, at least 27 days after emergence. This suggests that the fungus was unable to grow beyond cortical tissue without an external energy

source. Regardless of plant age (2–22 days) when exposed to sclerotial inoculum of *P. omnivorum* in containers, the root cortex sloughed 18–24 days and plants died 27–50 days after seedling emergence (Rush et al. 1984).

For many plants, infections must occur within a given temporal "window of susceptibility" for symptoms to develop and become severe. Susceptibility may coincide with the period of maximum growth of a plant part, for example, damping-off caused by *Pythium ultimum* killed snapdragon seedlings less than 15 days old but not seedlings greater than 20 days old (Populer 1978). Similarly, seedlings of beans, sugar beets, and many other plants are initially susceptible to damping-off caused by *Rhizoctonia solani* but become resistant about 21 days after emergence due to physiological changes (Bateman and Lumsden 1965). Susceptibility may change with age of an infected plant part, for example, the severity of Fusarium wilt on peas decreases with plant age due to restricted fungal growth in the xylem vessels rather than to decreased infections of the roots (Populer 1978). Susceptibility of potato tubers to *Streptomyces scabies* was restricted to a period of about 6 days, when the tuber had matured to the stage equivalent to development of three or four internodes (Lapwood and Adams 1973). Susceptibility may be bimodal, for example, potato tubers are susceptible to infection by *Fusarium caeruleum* during the first 2 months of tuber growth in the field and when tubers are in winter storage, but are relatively resistant shortly after the haulm is removed or senesces (Boyd 1967).

Crop yield losses may be related directly to the time that foliar symptoms appear relative to host plant phenology. When foliar symptoms appear late in the season, cotton lint reductions due to *Verticillium* wilt are small (Pullman and DeVay 1982). The effects of *Verticillium* wilt on yield are less severe when foliar symptoms develop during fruit development, because the plant has already slowed vegetative growth and partitioned more photosynthate to fruit for development (Pullman and DeVay 1982). For diseases that are not primarily systemic, such as *Phytophthora* root rots, early times of infection may indirectly reduce yield loss by lengthening the period over which symptoms increase in severity and alter host phenology to the extent that it results in yield reductions (Ristaino et al. 1989; Neher et al. 1993). For proper comparison, yield should be assessed for the same plants or plots on which disease severity was assessed (Kranz 1988).

Shoot symptoms should not be rated in isolation of root symptoms for root diseases, especially when interactions with plant nutrition and other microorganisms in soil may be involved. After confirmation of the cause of disease by examination of the roots or appropriate isolations, shoot symptoms can be relied on more exclusively for systemic diseases such as *Verticillium* wilts than for nonsystemic diseases such as *Phytophthora* root rots. In some cases where disease affects water transport in plants, the position and depth of lesions on roots may be a useful measure. Diameter and biomass of roots or maturation of leaves may be an important parameter

in assessment of root diseases which alter plant hormones. The time of symptom ratings should correspond to periods of host susceptibility or rapid epidemic development. Interpretation of disease symptoms should consider the environmental variables that may influence symptom expression and the growth of the host or pathogen such as temperature and humidity of the air and temperature and moisture of the soil.

5.4 Disease Assessment

Ideally, disease assessment methods should be (1) accurate, precise, and reproducible by many observers; (2) applicable over a range of conditions; (3) economical; and (4) simple (Kranz 1988; Berger 1988; Campbell and Madden 1990). Assessment methods and assessors can be evaluated in relation to a set of known or measured standards for accuracy and precision by linear regression (Amanat 1977; Kranz 1988; Campbell and Madden 1990). Reproducibility can be evaluated by correlation analysis. Inter-rater reliability can be evaluated by comparing the relative assessment of plant disease over a range of conditions by several observers (Shokes et al. 1987).

The purpose and scale of assessment must be considered when choosing an assessment method. Disease assessments may be conducted for a variety of purposes, including estimation of disease effects on yield, plant disease surveys, screening of fungicides, varietal trials, surveys to evaluate disease control methods, and forecasting (Large 1966). Methods for rating individual plants and plant populations may be different than for assessing disease for a large geographic region. Diagrammatic keys and scales (e.g., Horsfall and Barratt 1945) may be appropriate for rating individual plants and populations of plants, but remote sensing in surveys may be more appropriate for regional or continental estimations or comparisons.

It is also important to consider the plant organ(s) physiologically linked to symptom expression when selecting an assessment method. If fleshy or storage roots and fibrous roots are present or if woody and vegetative roots are present, such as with most trees, it may be necessary to evaluate disease on both types of roots. The focus of disease assessment may differ for plants for which the root (e.g., sweet potatoes, sugar beets, carrots) or the above-ground plant parts (e.g., tomatoes, oranges, corn, tobacco) are harvested as the yield component. For example, severity of soil rot or pox (*Streptomyces ipomoea*) symptoms on the fleshy storage roots of sweet potatoes has more effect on yield than symptoms on fibrous roots or shoots (Clark and Moyer 1988; Ristaino and Averre 1992). Cavity spot (*Pythium violae*) (Vivoda et al. 1991) and forked root (*P. irregulare* and *P. ultimum*) (Liddell et al. 1989) diseases of carrot affect primarily the roots and, thus, the economic yield. For *Phytophthora parasitica* var. *nicotianae* on tobacco, wilt symptoms on shoots directly affect the economically important leaves (Shew and Lucas

1991). In contrast, when fruit are harvested from above-ground plant parts, the severity of symptoms of shoots may influence yield more than the severity of root symptoms, for example *Phytophthora* root rot in processing tomatoes (Neher et al. 1993).

5.4.1 Illusions and Hazards of Disease Assessment

Errors in disease assessment may arise from perceptions of individual raters, from factors that influence the object rated, such as three-dimensional complexity of the sampling unit, size and shape of lesions, color of lesions in sampling unit and light conditions, and from the biological characteristic of a diseased plant such as root excision or defoliation due to disease. Errors in assessments are often cryptic and can lead to false impressions concerning the success or failure of management practices. Although not all of the illusions or hazards associated with disease assessments can be resolved simultaneously, awareness of potential problems can aid in improving the quality of assessments.

One concern is the accuracy of presenting maximum disease as 100%. Several authors (Kranz 1977, 1988; Sherwood et al. 1983) have discussed the problems with overestimation of disease. This illusion of disease assessment is compounded by the tendency to equate 100% with maximum disease severity. Some disease assessment scales such as the modified Cobb scale (Large 1966) have attempted to account for the fact that maximum disease severity may be less than 100%. In the modified Cobb scale for rust disease, 37% was chosen arbitrarily as the maximum proportion of leaf area that could be covered with rust pustules and this was labeled as 100% (Large 1966). Kranz (1977) also has suggested 37% as the maximum possible severity for many foliar diseases. Similar maximum limits have not been established with root diseases. There are also analytical problems in estimating the rate of disease progress when maximum disease is assumed to be 100% and it is actually lower (Analytis 1973, 1979; Park and Lim 1985; Neher and Campbell 1992). Incidence-severity relationships when incidence is 100% also cannot be assumed to be constant for all root diseases. Disease expressed as 100% incidence may be representative of severity for a systemic disease such as *Verticillium* wilt of cotton; however, 100% incidence may correspond to a severity rating of much less than 100% for a nonsystemic disease such as *Phytophthora* root rot on citrus.

Another hazard associated with assessment of symptoms of root diseases is that one of the main effects of root rots is the actual rotting of root tissues in soil. At first examination this statement may seem trivial; however, when a plant is removed from the soil, the symptom to be evaluated actually may be the amount of root tissue that is not present when it should be. The roots lost due to disease and subsequent decay in addition to normal turnover result in a smaller root system. The role of rotted or missing roots in disease

assessment is analogous to that of defoliation in the assessment of foliar diseases.

Another issue which poses difficulties in assessment of root diseases is the growth of the host during the period of time over which assessments are conducted. This difficulty is analogous to that of assessment of foliar diseases on a host that continues to add significant amounts of foliage during the course of an epidemic (Kranz 1988; Campbell and Madden 1990). What is needed is a measure or estimate of host growth or amount of host tissue present. If roots are removed from soil, root systems can be measured as discussed by Böhm (1979) and measures of root length can be made by direct measurements or by line-intersection, photoelectric, or digitizing methods (Newman 1966; Marsh 1971; Rowse and Phillips 1974; Tennant 1975; Voorhees et al. 1980; Campbell and Madden 1990). In addition, morphometric measurements of root systems provide information about root tissue type, and thus root function, which may relate to tissue susceptibility (Fitter 1982, 1987; English and Mitchell 1989).

5.4.2 Methodology

It is easier and less error-prone to measure incidence (Kranz 1988), therefore, it would be desirable to quantify incidence and indirectly relate it to disease severity. Incidence may be as informative as severity for some diseases, for example, those that (1) are systemic, e.g., fungal wilts, damping-off, and viral diseases (Seem 1984; Kranz 1988); (2) have little variation among severity levels within a sampling unit; or (3) the severity levels are low, i.e., in the early stages of epidemic development (Seem 1984). However, for other diseases such as *Phytophthora* root rot on citrus, incidence may be less informative and less accurate than disease severity (Horsfall and Cowling 1978; Seem 1984) in reference to the effects of disease on plant growth and yield. If relationships between disease incidence and severity are proven to be highly statistically significant by techniques such as correlation, linear regression, and nonlinear regression, it may be possible to quantify disease incidence and relate it to disease severity (Seem 1984). However, a variety of factors complicate the ability to quantify incidence with severity including differential tissue susceptibility, crop age or growth, and year-to-year and site-to-site variations in weather and environment (Seem 1984; Kranz 1988).

There are essentially two ways to measure disease intensity of root diseases: (1) destructively sampling "sacrificial" plants, or (2) assessing the intensity of root disease according to features of above-ground parts (Kranz 1988), i.e., visible symptoms or an expression of growth or stress. Both approaches have significant advantages and disadvantages (Table 1). In the first approach, it is essential to excavate the root systems of diseased plants several times during a growing season to assess the extent of diseased roots

accurately (Hornby and Fitt 1981). Because it is nearly impossible to excavate an entire root system, it may be necessary to dig plants with symptomless roots for comparison to root systems with disease symptoms.

The comparative excavation approach can be implemented in research studies by establishing experimental plots with paired rows of the crop. One row can be assessed for above-ground symptoms with nondestructive techniques and the other row for above- and below-ground symptoms by destructive sampling. In the row for destructive sampling, a randomly selected plant or quadrat of plants, which represent a small fraction of the total plants in a row, is removed at repeated intervals through the growing season to assess both shoot and root symptoms. Ristaino et al. (1989) and Neher and Duniway (1991) used this approach to characterize epidemics of *Phytophthora parasitica* on tomato. Campbell et al. (1980a,b) used a similar approach of removing only a small fraction of the entire host population to characterize epidemics of bean hypocotyl rot caused by *Rhizoctonia solani*. The level of impact that plant removal has on epidemic progress must be assessed. For example, above-ground symptoms can be compared between paired rows of destructively and nondestructively sampled plants to determine the effect of the destructive sampling on epidemic development.

Caution must be taken in inferring the extent of root disease by observing only foliar symptoms such as yellowing, epinasty, chlorosis, and wilting (Hornby and Fitt 1981). However, it is possible to indirectly assess symptoms of root disease by observing above-ground symptoms for plants with predictable patterns of symptom development on above-ground plant parts. For example, *Phymatotrichum* root rot of cotton initially causes the rapid wilting of upper leaves followed by wilting of lower leaves and plant death (Jeger and Lyda 1986). Also, statistically significant relationships have been established between incidence of above-ground symptoms of *Cylindrocladium* black rot of peanut and yield (Pataky et al. 1983). Disease incidence is rated on shoots throughout the season and roots are dug and evaluated at the end of the season (Pataky et al. 1984). Correlation of symptom severity on roots with above-ground plant symptoms may not be reliable until repeated research observations have been conducted and the associations confirmed (Kranz 1988).

An alternative to comparing root symptoms to shoot symptoms is to correlate root symptoms with above-ground plant growth, mass, color, leaf numbers, and respiration (Bald 1969). Assessment of host growth using leaf area (Bald 1969; Hornby and Fitt 1981) and biomass (Pullman and DeVay 1982; Ristaino et al. 1989; Neher 1990) are good estimates of growth and have been correlated with above-ground symptom severity. Leaf area can be measured using a variety of techniques including electronic leaf area meters, area diagrams, related area measurements, counting grids, and planimeters (Kranz 1988; Campbell and Madden 1990). Other estimates of host growth include plant height, canopy density and volume, percent ground cover, and plant growth stages (Berger 1988). Reduction in plant size is one of the first

indications of water deficiency in the field (Hsiao 1982) and is evident as an early symptom of *Phytophthora* root rots (Duniway 1977; Ristaino et al. 1989), *Fusarium* wilts (Gordon et al. 1990), and *Verticillium* wilts (Pullman and DeVay 1982). Reduction in growth rate was detected about 2 weeks prior to the appearance of foliar symptoms of *Verticillium* wilt on cotton (Pullman and DeVay 1982). Reduction in plant size indirectly reduces the plant's ability to produce photosynthate. Within fields, plant water or nutritional stress may be measured using spectral radiometers (Nutter 1989) and for larger areas, infrared photography (Powell et al. 1976; Toler et al. 1981; Jackson 1986).

5.4.2.1 Visual Estimates

General verbal or pictorial descriptive scales subjectively divide levels of disease severity into discrete categories (O'Brien and van Bruggen 1992a). These scales describe or illustrate plants with arbitrary amounts or types of disease symptoms (Lindow 1983). Diagrammatic scales are used to assess plant organs and field scales are used to assess whole plants or plots (Large 1966). Field scales must refer to the area covered by disease lesions and tissue chlorosis/necrosis caused by disease (Large 1966). The first standard area diagrammatic key used to assess disease severity was the Cobb Scale (1892) which differentiated five degrees of severity ranging from 1–50% leaf area covered with rust pustules on cereal leaves in Australia (Large 1966; James 1971; Horsfall and Cowling 1978). The McKinney Index (1923) was the first "numerical rating" assessment key. It was developed with categories ranging from 0–3, with 0 equal to no disease and 3 referring to abundant lesions. There are several problems associated with these types of scales including potential nonreproducibility of assessment by different evaluators (Lindow 1983; Sherwood et al. 1983), unequal application to symptoms of different cultivars, difficult interpolation of intermediate levels of disease severity (Lindow 1983), difficulty in evaluating how close estimated severity is to actual severity (Tomerlin and Howell 1988), and the potential requirement of nonparametric statistical analyses if intervals between categories are unequal. Accuracy may also be in question with use of categorical scales, as identified earlier, because observers tend to overestimate disease intensity (Berger 1980; Kranz 1988), especially at low levels of infection (Sherwood et al. 1983). However, accuracy and precision can sometimes be improved by experience (James 1971, 1974; Berger 1980; Lipps and Madden 1989).

The Weber-Fechner law states that visual acuity is proportional to the log of the intensity of the stimulus with poorest acuity occurring in moderate ranges (40–60%) of disease intensity (Large 1966; James 1974; Horsfall and Cowling 1978). Consequently, several scales have been developed using logarithmic scales, e.g., the James's keys (James 1971) and the Horsfall-Barratt (HB) scale. The HB scale comprises 12 classes: 0, 0–3, 3–6, 6–12,

12–25, 25–50, 50–75, 75–87, 87–94, 94–97, 97–100, and 100% diseased tissue (Horsfall and Barratt 1945). Berger (1980) proposed a finer division of the middle range of the scale into smaller intervals, but there is no evidence that observers can readily distinguish between these categories (Horsfall and Cowling 1978; Campbell and Madden 1990). Horsfall-Barratt scales were originally designed for foliar diseases, but the same concepts can be applied to root diseases given appropriate diagrams, e.g., corky root of lettuce (O'Brien and van Bruggen 1992a).

Some argue that logarithmic scales are appropriate for all diseases, even if the relationship between disease and loss is linear, because the eye reads in logarithms (James 1974; Horsfall and Cowling 1978), whereas others argue that a logarithmic scale is not appropriate for assessment of all diseases (Hebert 1982). Even under the guise of straight-percentage scales, observers have the tendency to cluster observations of disease intensity around "knots" or common values, for example, 0.0, 0.05, 0.1, 0.25, and 0.5 (Koch and Hau 1980). Percent scales can be used to describe a variety of assessment types, for example, the proportion of plants infected, area damaged, and proportion of roots or fruits affected. Categorical classes can be converted to straight-percentage scales by using the midpoint value of each category (Berger 1980; Campbell and Madden 1990). The advantage of this transformation is that straight-percentage scales can be analyzed using parametric statistical analyses.

There are a very few studies that compare disease assessment methods for accuracy, precision, or correlation with disease loss of root diseases (Forbes and Jeger 1987). A recent study compared assessment scales for corky root of lettuce by evaluation purpose and plant age for accuracy, precision, and correlation with disease loss (O'Brien and van Bruggen 1992a). A variety of experienced and inexperienced personnel evaluated the same plants for the percentage of the tap root area corked using three different scales. Two discrete interval scales (7-level scale for mature plants and 10-level for seedlings) were compared to a 12-level Horsfall-Barratt continuous interval scale. The scales tested were diverse with respect to simplicity (number of levels), realism (photographs vs. drawings), intended use (mature plant vs. seedling), choice of levels (objective vs. continuous interval), and statistical restrictions (discrete vs. continuous interval). No single assessment aid was identified as the best for all situations. The scales performed best for the phenological stage(s) for which they were originally developed and were most useful for predicting yield loss at phenological stages when disease severities fell within moderate ranges.

Forbes and Jeger's (1987) evaluation of a Horsfall-Barratt scale supported the agreement with the Weber-Fechner law in the sense that the greatest variances of disease assessment were within the 25–75% range. However, the variance was greater at 25% than at 50 or 75%, which would not be expected with the Weber-Fechner law. Symptoms on true roots were overestimated more than any other plant structure, including tubers.

This error was attributed to the nonuniformity of root shape and the scarcity of good scales for root diseases (Forbes and Jeger 1987). It is also more difficult to accurately assess three-dimensional than two-dimensional structures (Kranz 1988); therefore, there may be reason to rate individual roots or orders of roots (Fig. 5.1) or lesion placement, rather than a percent of the whole root system that has lesions or is affected by symptoms.

There are circumstances that complicate assessment of both root and shoot symptoms associated with root diseases. First, there are diseases where defoliation, root loss, and/or subsequent compensation occur as a result of severe disease. For example, the majority of isolates of *Verticillium dahliae* from cotton are defoliating pathotypes (Pullman and DeVay 1982). In contrast, a flush of new foliage may develop on processing tomato plants with severe symptoms of Phytophthora root rot late in the season (Ristaino et al. 1989). This new foliage, however, will never yield mature fruit. In other examples, adventitious roots may grow near the crown if other roots are severely pruned by rots and lesions, for example, by Phytophthora root rots of alfalfa (Stuteville and Erwin 1990), tomato (Blaker and Hewitt 1987a,b), and avocado (Kelman and Coffey 1985).

Second, under natural field conditions, complexes of pathogens may occur, making it difficult to differentiate symptoms of individual diseases. Examples of disease complexes include root rot of horseradish (Percich 1990), sudden death syndrome of soybean (Roy et al. 1989), bean root rot complex (Huber and Watson 1974), and numerous examples of fungal and nematode disease complexes including: (1) root knot nematode (*Meloidogyne* spp.) with *Fusarium* wilts on cotton (Powell 1971; Shepherd and Huck 1989), tomato, peas (Powell 1971); Phytophthora diseases on tomato (Neher 1990) and tobacco (Powell 1971); and *Pythium aphanidermatum* on chrysanthemum (Powell 1971); (2) cyst nematodes (*Heterodera*) with *Fusarium* wilts of soybeans and sugar beets; (3) sting nematodes (*Belonolaimus*) with *Fusarium* wilt of cotton and *P. aphanidermatum* infections of chrysanthemum; and (4) lesion nematode (*Pratylenchus*) with *Verticillium* wilts on cotton, tomato, eggplant, peppers, and potatoes (Powell 1971). Depending on the objective, the contribution of individual pathogens to the symptoms may be assessed or, from a holistic epidemiological perspective, the total amount of tissue with symptoms may suffice.

Third, there are situations where pathogen colonization has occurred, but no disease symptoms were visible, i.e., the completion of the incubation period has not occurred. This phenomenon has been reported for pathogenic *Fusarium* spp. (Gordon et al. 1989), *Verticillium dahliae* (Gerik and Huisman 1988), and *Verticillium albo-atrum* in moderately resistant alfalfa cultivars (Newcombe and Robb 1988). Using specific immunoenzymatic staining techniques, Gerik and Huisman (1988) found that *V. dahliae* was able to colonize the entire depth of the cortex of cotton roots without breaching the endodermis or infecting the stele. *Fusarium oxysporum* is primarily an

epiphytic colonizer, mostly confined to the root surface and outer cortex (Gerik and Huisman 1988).

Fourth, some fungi and nematodes may not cause much plant damage themselves, but cause damage indirectly by transmitting viruses. For example, infections by *Olpidium brassicae* on lettuce roots may be associated with symptoms of lettuce big vein (Grogan and Campbell 1966) and soilborne wheat mosaic virus is transmitted by the fungus, *Polymyxa graminis* (Wiese 1987). *Xiphenema index* is the vector of grapevine fanleaf virus (Pearson and Goheen 1988). Generally, *Xiphenema* and *Longidorus* spp. transmit nepoviruses and *Trichodorus* and *Paratrichodorus* spp. transmit tobamoviruses, i.e., tobacco rattle and pea early browning (Taylor and Brown 1981).

5.4.2.2 Electronic Techniques

Leaf area meters, video image analysis systems, multispectral radiometers, and remote sensing devices with infrared photography or satellite technology are among electronic devices that can be used for assessing plant disease. Leaf area meters are useful in assessing the area of detached leaves rapidly, which is an important variable in calculation of a leaf area index or other applications of plant growth measurement. Video image analysis systems are capable of assessing the area of diseased tissue based on color contrasts between healthy and diseased plant tissues. Images can be digitized and changes in color analyzed (Nilsson 1980; Lindow and Webb 1983; Berger 1988). Lens filters can be utilized to increase contrast between healthy and diseased tissue, for example, a red filter in sunlight under field conditions or a yellow filter with a light gray background for distinguishing diseased tissue with darkly pigmented lesions (Lindow 1983). Multispectral radiometers have been successfully used to quantify green leaf area gradients by measuring percent reflectance for a wavelength band of interest e.g., 800 nm (Nutter 1989). Chlorophyll pigments are the unit reflecting a wavelength band of 800 nm (Nutter 1989). In principle, healthy plants have greater reflectance than plants under stress or with disease symptoms of wilting, chlorosis, necrosis, or defoliation.

Black-and-white, color, color-infrared, and false-color-infrared photography have been used in remote sensing to detect plant disease at scales ranging from fields (Powell et al. 1976; May et al. 1985; Blakeman 1990) to large geographical regions (Berger 1988). The sharpest contrast in reflectance between soil and green vegetation is in the near-infrared band (700–950 nm) (Toler et al. 1981; Kranz 1988; Campbell and Madden 1990). Root diseases and their associated stress symptoms can be captured and viewed as color differences on film, sometimes before they are visible to an observer on the ground (Rundquist et al. 1984/1985; Kranz 1988). Plant canopies with reduced leaf water content and thus higher temperatures, caused by wilting, drought, salinity, or nutritional stresses, reflect less near-infrared energy

than healthy plants (Toler et al. 1981; Rundquist et al. 1984/1985; Jackson 1986; Kranz 1988). Canopy temperatures were 2.6–3.6°C and 3.3–3.5°C higher for sugar beets infected with *Pythium aphanidermatum* and cotton with *Phymatotrichum omnivorum*, respectively, than for healthy plants, even under conditions of water stress (Jackson 1986). Differences in reflectance, however, do not guarantee that a specific disease caused the stress. It is essential to accompany remote sensing with “ground truthing” (Horsfall and Cowling 1978; Kranz 1988). Once “ground truthing” is keyed to aerial photographs, surveys of expansive acreages can be conducted (Horsfall and Cowling 1978). Infrared photography may be useful for assessing systemic diseases but not for diseases with low severity that begin in the lower canopy hidden by upper healthy foliage (Berger 1980), or for diseases that develop before the leaf canopy is full, because exposed soil will further reduce reflectance in excess of diseased tissue (James 1974). Reviews by Jackson (1986) and Toler et al. (1981) are recommended for additional information.

Use of aerial photography has been reported more often for foliar than root diseases (Toler et al. 1981). *Phymatotrichum* root rot (*P. omnivorum*) on cotton and take-all (*Gaeumannomyces graminis*) on winter wheat are root diseases that have been assessed successfully with aerial photography. Sharp contrasts between diseased and healthy cotton plants were apparent because plants died quickly after the onset of symptoms (Toler et al. 1981). With take-all in the UK, aerial photography has been used since the 1980s to provide a visual record of case histories which demonstrate how soil and cultural interactions affect the development of the disease (Blakeman 1990). Aerial photography has also been used to detect Fusarium wilt on pear (Haglund and Jarmin 1978) and aerial photography may be useful for orchards and forests where disease development can be monitored and mapped on an annual basis (Toler et al. 1981). For example, Wallis and Lee (1984) used aerial photography to detect centers of root disease in Douglas-fir stands. However, successful assessments were dependent upon stand age, i.e., older stands with large openings caused by diseased or dead trees in the stand were easier to discern than younger stands with less distinct stand contrasts (Wallis and Lee 1984). Also, it was impossible to distinguish the two etiological agents, *Phellinus weirii* or *Armillaria ostoyae*, from aerial photographs using color or color-infrared film. However, distinct red or yellow foliage was discernible on both color and color-infrared film (Wallis and Lee 1984). MacNish and Lewis (1985) compared the relative ease and accuracy of aerial mapping, ground area mapping, and ground-based scoring methods for determining the extent of Rhizoctonia patch in cereals relative to yield. The three methods were highly correlated, but ground-based scoring was quicker, more convenient, and related better to yield losses than either aerial or ground-based mapping methods (MacNish and Lewis 1985) and was most likely more cost-effective (Toler et al. 1981).

5.5 Sampling Considerations

The challenge in sampling for disease assessments is to obtain a representative and statistically valid sample of the population on plants for an affordable cost with the greatest likelihood of preserving the integrity of the host population and, thus, the epidemic of interest. The questions that must be answered are “how many samples should be taken?” and “how should the samples be taken?” To answer these challenges, sampling must balance statistical, biological, and economic considerations.

5.5.1 Sampling Pattern

Most root diseases have an aggregated spatial pattern in the field (Campbell and Noe 1985; Gilligan 1988; Campbell and Madden 1990; Chap. 7, this Vol.). In simulation studies with aggregated disease patterns, systematic sampling provided better estimates of disease incidence than did simple random sampling (Lin et al. 1979). In a systematic sample, the number of so-called sampling “arms” or, basically, the number of changes in direction during a systematic sampling path through a field, is important to sampling success. As a result, a zigzag pattern, a W-shaped or diamond-shaped path is recommended for moving through a field to assess a root disease. Thus, once a random starting point is selected in a field or a stratum (e.g., x paces from one edge of the field and y paces from the adjacent edge of the field or a neighboring stratum), samples can be taken or sites of assessment selected every k paces or units along the selected sample pattern so that a large portion of the field is covered by the time n sample units (the number of sample units desired) have been assessed.

In another simulation study, stratified random sampling had a lower error rate or bias (difference between true and estimated mean) than other designs when incidence of disease was low (<10%) (Delp et al. 1986a). To determine inoculum density of soilborne pathogens, which also occur in aggregated patterns, a systematic pattern of sampling is also usually recommended (Goodell and Ferris 1981; Hau et al. 1982; Mihail and Alcorn 1987) and stratification of fields may improve estimates of disease at low incidence levels. A stratified random sampling design is implemented by dividing the field into equally sized sectors and sampling at randomly or systematically selected locations within each sector. This method has been viewed as cumbersome in the past, but is made simpler with a computer program called “Field Runner” designed for use on a portable personal computer, which directs the evaluator to specific locations in the field (Delp et al. 1986b).

5.5.2 Sample Numbers and Costs

The number of samples (n) taken for a disease assessment determines the quality or reliability and the cost of the assessment. Three factors that must be considered to determine sample number are the statistical estimates of the mean and variance obtained in a preliminary sample, the biological characteristics of the pathosystem, and the resources available for sampling. Most sampling texts have excellent presentations concerning the ways in which to estimate sample number based either on statistical criteria such as mean, variance, and desired reliability or on statistical criteria and resource allocation (= costs). Several sources of such information provided by biologists should be consulted for readable presentations of such considerations (Karandinos 1976; Ives and Moon 1987; Kranz 1988; Campbell and Duthie 1989; Campbell and Madden 1990). However, few if any, strictly statistical approaches to sampling design make allowances for or provide recommendations how to account for the very real biological differences of sampling such as those that are encountered in assessment of root diseases, particularly when destructive sampling is necessary.

5.6 Recommendations

Several preliminary steps must be taken before successful disease assessment can be completed. First, be familiar with the symptoms of the disease(s) of interest. Second, define the objectives and goals of the disease assessment and determine the desired detection level. An assessment scale should be chosen to fit the detection level and maximum level of disease observed. Selection of an appropriate scale may require knowledge of the relationship of disease development to such factors as plant phenology and climate. Third, decide whether incidence and/or severity of disease is important in relationship to the study goals. Fourth, choose the plant organs that will be evaluated. This choice depends upon the disease and the study objectives, i.e., shoot symptoms may be more important for assessment of effects on yield for some diseases, whereas assessment of root and shoot symptoms may be necessary to determine physiological relationships that occur within a diseased plant. Once the choice of plant organs is made, the experiment can be designed to permit the type of sampling required to assess symptoms on the chosen plant organs at the desired frequency.

Estimation of disease intensity should begin early in disease development, generally when symptoms or signs first appear so that several estimates in time can be made. Berger (1980) suggested that observations be made every one-half incubation period with more assessments during rapid stages of epidemic development. The application of this guideline may be complicated if root and shoot symptoms are rated because of the potential

difference in time of occurrence or detection of the incubation period for visible symptoms on shoots and roots. Assessment on a series of dates is more beneficial than a single rating at the end of a growing season (Watson et al. 1990). Also, with many statistical procedures, there is a gain in accuracy when the number of observations is large ($n > 15$) (Berger 1980).

It is advisable to use a scale with equal divisions, whether on a linear or logarithmic scale (James 1975; Kranz 1988). Such a scale makes the decision process during the disease assessments easier. Also, data derived from a scale with equally spaced divisions can be analyzed easily by use of parametric statistical analyses such as analyses of variance and regression.

Due to the potential influence of diurnal weather cycles, assessment of shoot symptoms associated with root diseases should be made at a similar time of day, preferably early in the day, because evapotranspiration demand can influence the expression of wilt symptoms. Sun angle and brightness may also influence the perception of color differences associated with disease symptoms.

Selection of an appropriate sampling design for assessment is critical. Systematic designs are preferred for aggregated diseases such as root diseases. A stratified random design can be used for diseases with a variety of spatial patterns (Delp et al. 1986a). Sample number should be based on information from preliminary samples and will be determined by balancing error and cost considerations.

The record of intensity of disease at each assessment should be accompanied with data on crop growth stage, the plant organ assessed (James 1974; Watson et al. 1990), the spatial pattern of disease (Watson et al. 1990), and the relevant sampling information (Campbell and Madden 1990). Also, environmental variables that may correspond to epidemic development, such as soil temperature and moisture, should be monitored. This information will allow meaningful evaluation of assessment scales and comparisons between epidemics.

Disease assessment should be followed by isolation of the pathogen of interest to confirm the cause of symptom development. Pathogen isolation and careful evaluation of all symptoms will permit (1) accurate diagnosis; (2) identification of multiple pathogens and diseases which may accentuate or diminish the symptom expression of the disease of interest; and (3) detection of interfering mineral deficiencies or toxicities. When a complex of diseases is present, initially assess the total integration of diseases on the plant(s), followed by assessment of effects of individual diseases as much as possible (Berger 1988). If multiple plant organs are effected by disease(s), assess one disease on one organ at a time; the error attached to any particular assessment will be less than when organs are evaluated collectively (James 1974). For survey or regional studies where remote sensing is used, "ground truth" activities should be adequate to provide evidence of the causal agents.

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