INFECTION PROCESS - PLANT DEFENSES

Plant pathogens fall into two broad categories: necrotrophs (those that kill plant cells before parasitising them), and biotrophs (those that obtain nutrients from living cells). Failure of pathogens to invade suitable host cells (dead in the case of necrotrophs; alive for biotrophs) will prevent them from infecting the host and the plant will be *resistant*. Additionally, the establishment of a parasitic relationship is dependent on the responses of the plant under attack.

Resistant hosts prevent or slow the development and reproduction of the majority of pathogen propagules that they come into contact with. Resistance can be expressed at many stages in the infection process, from inhibition of propagule germination and penetration, to the restriction of colony development after the pathogen has become established. The defence barriers erected by plants are a co-ordinated system of molecular, cellular and tissue-based responses to pathogen attack.

Protection from a pathogen's initial invasion is achieved via passive defences, such as physical and/or chemical barriers.

Physical barriers largely involve properties of the plant surface, that is, the cuticle, stomata and cell walls. Pathogens produce a range of cutin-degrading enzymes, which are often crucial to the successful penetration of the plant tissue. The thickness of the cuticle, the presence of secondary cell wall, and the size of stomatal pores can all affect the success with which a pathogen invades a host. Some plants invest in very thick walls and/or cuticles, and bark (where present) can also provide a physical impediment to infection. The vertical orientation of leaves can also add to plant resistance, by preventing the formation of moisture films of the leaf surfaces, inhibiting infection by pathogens reliant on water for motility.

Chemical barriers include compounds, such as "phytoanticipins", that have antimicrobial activity and compounds that affect the vectors of plant viruses. Phenols and quinones are two classes of antimicrobial compounds produced by some plants Inhibiting compounds may be excreted into the external environment, accumulate in dead cells or be sequestered into vacuoles in an inactive form. The young fruit of numerous plants (e.g. mangoes, avocado) contain antifungal or antimicrobial compounds that are gradually metabolised during fruit ripening, making unripe fruit less susceptible to disease than ripe fruit. Lactones, cyanogenic glucosides, saponins, terpenoids, stilbenes and tannins are also plant-produced compounds associated with pathogen resistance. Saponins are a class of phytoanticipins that destroy membrane integrity in saponin-sensitive parasites, and which are stored in an inactive form in the vacuoles of the plant cell, becoming active when hydrolase enzymes are released following wounding or infection. Some pathogens are able to release enzymes that detoxify plant saponins, making them insensitive to this line of defence. Conversely, resistance of some plants to specific pathogens is the result of an insensitivity to pathogen-produced host specific toxins. Resistance genes may encode an enzyme that converts the toxin into a non-toxic derivative or the absence of a receptor to the toxin. Another group

of defensive compounds are the plant defensins, which interfere with pathogen nutrition and retard their development. Secreted defensins can create an antimicrobial microenvironment for germinating seeds and accumulated defensins can provide defence against insect-transmitted viruses in flowers, leaves and tubers. There are also proteins, both constitutive and induced that play a role in plant defence.

PATHOGEN RECOGNITION

Plants use a vast array of signals originating from micro-organisms and the environment to recognise pathogens and elicit plant defence responses. **Non-specific elicitors** of biotic and abiotic origin induce host defences in a broad range of host species. **Abiotic elicitors** such as heavy metal ions or UV light can induce stress responses in exposed tissues, which may provide an additional barrier to invading pathogens or alternatively, increase the plant's susceptibility to infection. **Biotic elicitors** include cell wall fragments released from fungi and bacteria, hydrolytic enzymes of plant or pathogen origin, some peptides, glycoproteins and polyunsaturated fatty acids. These elicitors induce defence responses in a range of host species. Often, non-specific elicitors act as a general indication that the cell has been damaged in some way (for example, the release of fragments of the host's own cell wall can elicit defence responses).

Specific elicitors enable defence against a very specific pathogen, and are conditioned by *avirulence genes in that pathogen*. Avirulence genes determine the pathogen's host range, but are only able to function in the presence of another set of genes, the 'hypersensitive response and pathogenicity' (Hrp) gene cluster. Some Hrp gene products are involved in disguising the pathogen from host recognition, thus playing a role in both virulence and avirulence.

For a biotroph to form a successful infection, it must establish a basic **compatibility** with its host. The pathogen may also produce compatibility factors that delay, avoid or negate recognition by a normally resistant host plant. Virulent strains appear to be able to suppress the resistance mechanisms of the host, but are not able to halt resistance responses once they are activated. **Incompatibility** between a host and a pathogen results in the recognition of the pathogen and activation of defence mechanisms, while compatibility results in infection.

Specific elicitors are encoded by **avirulence** genes, and these peptides are believed to bind to receptor peptides, encoded by host resistance genes. Recognition of the avirulence gene products by the host triggers signal transduction pathways that cause a massive shift in gene transcription and plant cell metabolism, and local and systemic signals are released that prime the rest of the plant against further infection. The presence of non-specific elicitors, such as the release of host and pathogen wall fragments, during this process may amplify the defence response.

Host-parasite specific resistance is determined by the interaction of between products of pathogen avirulence genes, Specific elicitors and products of host resistance genes. The defence responses of plants can be very rapid. Host gene expression begins within minutes, or even seconds, of exposure to elicitors or pathogens. A diverse range of elicitors can induce a common set of responses in the host, suggesting that second messengers are involved in the signalling pathway between pathogen attack and host response.

RAPID ACTIVE DEFENSES

Almost every host-parasite interaction is unique in the details of the activation, localisation, timing and magnitude of the defence responses.

At the membrane

The host membrane appears to be involved in the earliest stages of pathogen recognition and signal transduction. A **change in membrane permeability** after exposure to a pathogen causes fluxes in ions, such as K+, H+ and Ca2+, and results in changes to gene activation and the triggering of the defence responses. Also at the membrane, the '**oxidative burst**', which involves the generation of reactive oxygen species, such as hydrogen peroxide, triggers signals that affect gene expression, cross-linking in the host cell wall and initiation of later defence responses. The reactive oxygen species at the site of infection are also produced in quantities capable of killing micro-organisms directly.

At the cell wall

Preparations for the **reinforcement of the cell wall**, which can improve host resistance, begin very quickly after a pathogen attempts to penetrate a host cell. This is characterised by an intensification of cytoplasmic streaming and the accumulation of host cytoplasm around the site of attempted penetration. The cytoplasmic aggregates are thought to contain cellular apparatus for the synthesis of cell wall fortifications. If the host cell can repair and reinforce its cell walls quickly enough, it might reduce the penetration efficiency of the pathogen. Several types of reinforcement are produced by host cells. A papilla is a deposit of callose, silicon, lignin and proteins between the cell wall and cell membrane, directly below the point of attempted penetration, while lignitubers are lignified callose reinforcements that ensheath invading hyphal tips.

Hydroxyproline-rich glycoproteins are structural cell wall proteins involved in secondary cell wall thickening. The expression of genes governing their production is activated ahead of invading hyphae, reinforcing walls. Cross-linking of hydroxyproline-rich glycoproteins caused by the release of hydrogen peroxide in the oxidative burst also reinforces cell wall compartments. Rapid deposition of lignin and suberin following infection also increases resistance to pathogens in many plants. Lignin can also bind to hyphal tips and bacteria, physically restraining them and restricting the diffusion of their enzymes and toxins into, and the extraction of water and nutrients out of, the host cell. Cell

wall reinforcements tend to be larger and more quickly formed in resistant hosts than in susceptible hosts, and inhibition of the production of callose or lignin synthesis by the pathogen enhances its penetration efficiency.

The hypersensitive response

Hypersensitive cell death is another widespread mechanism used by hosts to prevent the spread of a pathogen. Infected cells and those surrounding them "suicide", preventing further spread, and in some cases, killing the pathogen. It is often associated with the initiation of other responses, such as lignification and the synthesis of anti-microbial compounds. The success of hypersensitive cell death as a resistance mechanism depends on the nutritional requirements of the specific pathogen and the timing, magnitude and location of the host response.

Antibiotic compounds

Phytoalexins are low molecular weight antibiotics produced by many (but not all) plants in response to infection. There are many biotic elicitors of phytoalexin production, such as cell wall components, as well as abiotic elicitors, such as heavy metals and ultraviolet light. Phytoalexins inhibit the growth of bacteria and fungi *in vivo* and *in vitro*, and production of these antibiotics during an infection can induce resistance to subsequent infections by that pathogen. Over 350 phytoalexins are known in over 100 plant species. They include pterocarpans, sesquiterpenes, cryptophenols, isocoumarins, isoflavenoids, and others. Phytoalexins may be produced by any part of the plant, although different phytoalexins can accumulate in different organs. Generally, related plant species produce structurally-related phytoalexins, and many produce more than one, enabling the plant to present a toxic cocktail to invading pathogens. Phytoalexins are produced in cells surrounding an infection site and delivered to the infected cell packaged in lipid vesicles, creating a toxic micro-environment in the infected cell and, hopefully, preventing disease establishment. Phytoalexin accumulation is often associated with hypersensitive cell death, although only living cells can synthesise phytoalexins. Some plants can also sequester phytoalexins into vacuoles as stores of inactive sugar-conjugates, which can be cleaved and released quickly if initial defence responses are unsuccessful.

DELAYED ACTIVE DEFENSES

Delayed active defences include containment of the pathogen, wound repair, expression of pathogenesis-related proteins and the acquisition of systemic resistance. These mechanisms restrict the spread of the pathogen after infection is established and contain the damage to host tissues.

Physical responses

The ability to repair wounds can help protect the plant from further infection by other, opportunistic pathogens. A secondary meristem in fleshy tissues, fruits, roots and bark, the cork cambium, can produce cork cells, which have thick,

suberised walls. These cells can create a barrier to further colonisation by the pathogen and, in some cases, develop an abscission layer around the site of infection, causing the infected tissue separate from the healthy tissue. Wounded tree trunks often secrete protective gums that seal the wound from further infection. The formation of tyloses, ingrowths if the protoplasm in xylem parenchyma can also restrict the spread of pathogenic propagules in the xylem, although they also tend to reduce the movement of water through the vessels, causing water stress in the plant.

Pathogenesis-related proteins

There is a range of novel proteins synthesised in response to infection, many of which have b-glucanase, chitinase or lysozyme activity. Some **pathogenesis-related proteins** disrupt pathogen nutrition. The presence of low levels of these proteins in healthy plants suggests that they might have other roles in plant growth and development aside from disease resistance. Chitinase and glucanase accumulate in the vacuoles, and glucanase is also sometimes secreted into the intercellular space. They dissolve the fungal cell wall, fragments of which then elicit hypersensitive cell death. The breakdown of the vacuole during decompartmentalisation of the cytoplasm results in a flood of hydrolytic enzymes, which have antiviral, antifungal and antibacterial activity. The accumulation of pathogenesis-related proteins peaks around 7-10 days after initial infection. The presence of these proteins *before* infection increases the plant's resistance to pathogens, as in the case of systemic acquired resistance.

Systemic acquired resistance

Systemic acquired resistance, or induced resistance, is characterised by the increased resistance of a plant to a wide range of pathogens following infection by one pathogen. It is therefore fundamentally different from the specific antigen-antibody mechanism of resistance seen in the immune response of mammals. Rather than providing immunity *per se*, systemic acquired resistance reduces the severity of later diseases. The development of systemic acquired resistance usually requires the development of a slowly expanding necrotic lesion and other localised responses to infection, the release of a phloem-translocated signal originating from the infection site, and the subsequent priming of the plant against further attacks, allowing a more rapid response in the case of future infections. The nature of the signal that triggers systemic acquired resistance is as yet unknown, and is likely to be a complex signal transduction pathway mediated by a number of stress signals. Salicylic acid plays a key role, via interaction with salicylic acid-binding proteins that can cause build-up of reactive oxygen species or activate gene expression. The levels of salicylic acid increase around necrotic lesions and remain high in plants that have acquired resistance. It is not, however, salicylic acid itself that acts as the signal that is translocated systemically throughout the plant.

Co-ordination of defence responses

The success of defence responses is increased if activated in combination. Passive mechanisms, coupled with rapid active responses and slower follow-up defences provide a broad defence front to the plant. The specific interaction between host and pathogen is of course crucial to the success of the plant's resistance or the pathogen's invasion, and is mediated by the many pathways involved in producing or detecting elicitors, enhancers, suppressors and secondary signals.

INFECTION PROCESS: DISEASE DEVELOPMENT

The amount of disease that develops in a plant community is dependent on properties of the host, the pathogen and the environment. The environment can affect both the susceptibility of the host (e.g. by creating stress in the plant) and the activity of the pathogen (e.g. providing moisture for spore germination). The pathogen and the host can affect each other's performance. The plant can also change its environment, by creating a microclimate around it.

Table 1. Factors that affect disease development.

Pathogen	Host	Environment
• Presence of pathogen • Pathogenicity • Adaptability • Dispersal efficiency • Survival efficiency • Reproductive fitness	• Susceptibility • Growth stage & form • Population density & structure • General health	• Temperature • Rainfall / Dew • Leaf wetness period • Soil properties • Wind • Fire history • Air pollution • Herbicide damage

THE PATHOGEN

The presence or absence of a pathogen is the main factor that determines whether disease occurs. Introduction of a pathogen to an area from which it has previously been absent can cause major outbreaks of disease in plant communities. The amount of disease that develops is often determined by the pathogenicity of the main pathogen. The term pathogenicity relates to both the virulence (infection ability) and the aggressiveness (the vigour of the infection) of the pathogen. Pathogenicity is dependent on the pathogen's reproductive, dispersal and survival fitness.

The **adaptability** of the pathogen is also important in determining its ability to infect resistant hosts or to survive changed environmental conditions. Adaptability is determined by the pathogen's genetic flexibility and reproductive efficiency. The spread of a disease and the formation of epidemics is reliant on the pathogen's ability to **disperse** rapidly over long distances. The spores of cereal rusts, for instance, can be blown over vast

distances in a few days, while soil-borne pathogens have little scope for extensive spread. For a pathogen to cause disease in successive seasons, it must be able to **survive** the intervening time. Some pathogens form spores or sclerotia that can survive in the soil for years, while others colonise alternative plant species until the season of their primary host comes around again. Beyond the mere presence of a pathogen, the **number of infective propagules** available to infect plants is a crucial factor in determining the amount of disease that develops. Generally, as the number of propagules increases, the level of disease increases, levelling off when the amount of disease reaches very high levels and there are few uninfected plants available. The survival of propagules, and therefore the number of propagules available to cause disease, is heavily influenced by environmental factors.

THE HOST

The development of disease in a plant community relies on the presence of individual hosts that are **susceptible** to that particular pathogen. If the majority of the population is susceptible to the pathotypes of a pathogen in the vicinity, an epidemic can occur.The best way of controlling disease is by planting species or cultivars that are not susceptible to pathogens of that area. The occurrence of disease can also be influenced by the host plant's **growth stage and form**. Some diseases are common in seedlings, while others are typical of mature plants. The growth stage of the population can also affect the microclimate around the plants; for example, the humidity and sunlight levels under the canopy. The **population structure and density** will also affect the development of disease in a plant community. The density of the main host species and the proportion of other plants that are not hosts within the community will determine the rate and extent of epidemic development. Crop plants tend to be densely planted, with no other species in amongst them, making them more susceptible to rapid spread of disease. Extensive, dense plantations can host spectacular epidemics, particularly if a new pathogen is introduced to the area. In addition, the general **health** of the host plant before infection is important in determining the success of a disease. Necrotrophs do well on poorly growing plants, while biotrophs thrive on a healthy host plant.

THE ENVIRONMENT

The presence of a pathogen against a particular plant will generally not cause serious disease unless the environmental conditions are favourable. This includes the aerial environment and the soil (*edaphic*) environment. Human attempts at controlling disease usually involve manipulating the environment in some way. For example, breeding wheat cultivars to tolerate dry conditions allows Australian farmers to plant the crop in areas that are not favourable for pathogens such as powdery mildew and leaf rust. Properties of the **aerial environment** that influence disease development include moisture levels, temperature and pollution.

Moisture is particularly important to pathogenic bacteria and fungi. Rain splash plays an important role in the dispersal of some fungi and nearly all

bacteria, and a period of leaf wetness is necessary for the germination of most airborne spores. By using water for dispersal, propagules are dispersed at a time when they are likely to be able to germinate as well. Because the process of germination and infection takes time, the duration of leaf wetness also influences the success of the infection. The duration necessary for infection varies with temperature. Usually, a longer period of leaf wetness is needed to establish an infection in cooler temperatures, as germination and infection are generally accelerated in warmer conditions.

Temperature also affects the incubation, or latent, period (the time between infection and the appearance of disease symptoms), the generation time (the time between infection and sporulation), and the infectious period (the time during which the pathogen keeps producing propagules). The disease cycle speeds up at higher temperatures, resulting in faster development of epidemics. The period of leaf wetness, combined with temperature information can be used to predict outbreaks of some diseases (infection periods) and be used to time preventative treatments, such as spraying. A recently recognised aspect of the aerial environment that can influence disease in plants is air **pollution**. A high concentration of pollutants can affect disease development and, in extreme cases, damage the plants directly by causing acid rain.

The **edaphic (soil) environment** affects soil-borne diseases, largely by determining the amount of **moisture** available to pathogens for germination, survival and motility. Germination and infection success also rely on the temperature of the soil. The **fertility** and **organic matter content** of the soil can affect the development of disease. Plant defences are weakened by nutrient deficiency, although some pathogens, such as rusts and powdery mildews, thrive on well-nourished plants. Other diseases thrive in soils that are specifically low in organic matter.

INTERACTION BETWEEN FACTORS

The pathogen, the host and the environment interact, usually in ways that are difficult to quantify and predict. Control measures can include sowing of a crop species early, to avoid exposing seedlings to a disease during the time of year that provides the best environmental conditions for the pathogen.

INFECTION PROCESS: EPIDEMIOLOGY

Prediction of disease outbreaks enables the effective use of control measures, such as chemical or biological treatments, the prediction of crop yields and of the market potential for that crop. Disease forecasting involves the use of **weather data** and **biological data** to predict disease incidence. Usually, disease forecasting is only performed on economically important diseases, and as a method of cost reduction. If controlling a particular disease involves an expensive or time-consuming treatment, being able to predict outbreaks of the disease allows the treatment to be timed correctly, increasing its effectiveness, and reducing the cost compared to repeated treatments. Because environmental conditions vary from season to season, disease

forecasting is necessary to predict the chance of disease in a certain set of conditions.

Disease can be forecast using computer modelling and empirical correlations relating to weather conditions, levels of inoculum, test plots and site factors and the predictions can then be communicated to growers. **Computer modelling** of plant diseases uses systems analysis to accumulate all the factors that affect the development of a certain disease into a computer-based model, and make predictions of disease under different environmental conditions. A disease needs to be well understood in order to formulate an accurate model, and models based on diseases that we know little about are generally not very accurate. The more straightforward approach of developing **empirical correlations** between particular weather factors and disease has had considerable success. This does not attempt a complete modelling of all factors involved in a disease, but only those most important in affecting the disease. The accuracy of the model can then be measured statistically by comparing its predictions to what actually happens.

Monitoring the weather is the most important consideration in disease forecasting, because of the overriding effect that weather has on disease development. While broad scale weather data has been used for disease forecasting, it is well known that the microclimate within the crop has a more direct impact on disease. Devices have been developed to monitor microclimate factors such as duration of leaf wetness and temperature, and with time, they will be affordable and accurate enough for widespread use on individual farms. Synoptic weather forecasting charts can be used to predict 'critical periods' - the occurrence of conditions favourable for disease development - so that farmers can spray their crop before it happens. There are now several self-calculating disease forecasting monitors available commercially that use environmental data and past season data to predict outbreaks of particular diseases.

Some disease forecasting methods are based solely on **monitoring inoculum** levels, often as indicated by the amount of disease already present. This method can be successful when disease is developing steadily under relatively uniform or predictable weather conditions, but not for diseases that can spread explosively in favourable conditions. Monitoring the amount of disease present can indicate whether the amount of disease is likely to exceed a certain threshold, at which point control measures become economical. There are numerous methods of directly monitoring the concentration of spores in the air as an indication of the chance of disease. Trapping vectors of diseases can also be useful in predicting the occurrence of viral diseases. In addition, estimation of populations of soil-borne pathogens by examining soil samples is necessary for predicting the outbreak of the diseases they cause. Monitoring systems can be combined with **data specific to the site and the crop**, such as soil type, topography and irrigation levels, in order to increase the accuracy of predictions.

Test plots (or trap plots) of susceptible cultivars can be planted throughout a cropping area to give early warning of the arrival of inoculum or disease

vectors. Alternatively, inoculation of the test plot with the pathogen can give an indication of favourable environmental conditions for disease development. Test plots are also useful for monitoring the occurrence of minor diseases on new cultivars.

The formation of a prediction is useful only if it can be **communicated** to the growers who will be affected by it. General warnings for areas are broadcast over the radio or internet. Predictions based on monitoring by individual farmers or groups of farmers in an area remove the need for widespread communication systems. Computerised decision support systems based on local monitoring can educate and empower farmers when making decisions about their crops.

INFECTION PROCESS: DISEASE ASSESSMENT

Critical information in the assessment of disease is the **amount** of disease that is present. This can be measured as the proportion of a plant community that is diseased (**disease incidence**) or as the proportion of plant area that is affected (**disease severity**). Often, disease has to exceed a certain threshold before it reduces the yield of a crop, but it is usually difficult to accurately estimate the yield reduction caused by a specific disease. For example, many diseases occur on senescing tissue that would not have contributed to the yield anyway. Easier diseases to assess are those that kill whole trees in orchards or plantations, and those that destroy the actual harvested product, such as fruit or grain.

Disease and crop loss assessments are necessary for evaluating the economic impact of a disease and the benefit of particular control strategies. There is no point in implementing a control measure if it will cost more that the increased crop yield will return. The growth of the crop, its yield potential, the development of the disease and its impact on yield all have to be measured to predict the impact on yield of particular levels of disease. This information can be combined with predictions of likely disease levels to determine whether preventative treatments are worthwhile.

In the past, most crop loss assessment has been qualitative, producing vague, inaccurate and sometimes misleading data. One major problem with this is the complex nature of disease development. Rarely can disease be attributed to just one factor. Assessment of the effect of disease on crop yield normally involves five steps:

- developing a descriptive growth stage key for the particular crop species
- developing methods to assess the incidence and severity of disease
- developing statistically sound methods of sampling crop populations for assessment of the amount of disease
- estimating the negative impact of particular levels of the disease on crop yield and quality, and
- evaluating the economic benefit from various methods available for reducing the amount of disease.

ASSESSMENT OF CROP GROWTH AND DEVELOPMENT

The first step to quantify the effect of disease is to develop a key that describes the growth and development of healthy plants during the growing season. It should describe development, either from sowing to harvest, in the case of annual plants, or from season to season in perennial plants. Details drawings or photographs, showing characteristics of the various stages of development, including leaf formation, flowering, fruiting and senescence are needed. Standardised growth keys have been developed for a number of crop plants, enabling comparison between different countries and different conditions.

ASSESSMENT OF DISEASE INCIDENCE AND SEVERITY

Disease assessment methods need to easily provide objective measurements for a specific growth stage of a crop so that data from different sources is comparable, and provide an adequate sample of the crop for assessment. Whether it is disease incidence, or disease severity, or both, that are measured, depends on the nature of the disease. An "all or nothing" disease, for example, that inevitably kills any plant it infects, could be measured just by counting the number of plants that infected (disease incidence). However, in the case of a disease that causes varying degrees of damage to plants throughout the crop, a more complex measurement is needed, that assesses disease severity. The disease incidence for biotrophic pathogens can be measured by counting the number of plants, leaves, flowers etc that are infected, but the disease severity is assessed by estimating the proportion of total photosynthetic area that is diseased. While this is generally less precise and less controllable than counting individual plants, it is usually a better predictor of crop loss. Because judging the proportion of diseased leaf by eye is unreliable, **disease assessment keys**, showing different disease severities as blackened areas, have been devised for various crops.

To produce a disease assessment key, the development of disease over the whole disease cycle and at different stages of plant growth must be studied to make prototype standard diagrams and/or descriptions. The accuracy of the key then needs to be tested, by assessing disease severity in the field using the key, and then assessing the same samples using accurate measurement techniques in the laboratory. There are also computer-programs designed to train observers in disease severity assessment, by presenting images of diseased leaves, which the observer assesses, and comparing their result with the known level of disease in the diagram. This aims to reduce variation in results caused by different observers.

These disease assessment methods will only be accurate if performed on a representative sample of the crop. Samples of crop units (plants, leaves, fruit etc) can be taken randomly from a crop, or standard quadrats can be placed in the crop and all plants within the quadrat assessed. In a test plot, a part of the plot is usually assessed for disease. Taking samples only from the edge of a plantation will not necessarily be representative of the bulk of the crop. To determine how many samples need to be taken, it is possible to sample a

number of times with progressively more samples, and find the point at which the standard error is low. A disease that is uniformly spread throughout a crop will require fewer samples for an accurate assessment than a disease that has a patchy distribution throughout the crop.

ASSESSMENT OF CROP LOSSES

Once the amount of disease has been determined, the next step is to assess, either experimentally or statistically, the effect of different levels of disease on the crop yield. Experimental assessment involves setting up test crops in which the level of disease is controlled. Monitoring crops with different levels of disease allows the comparison of epidemic progress and crop yield under different disease conditions. A relationship between disease parameters and yield can then be formulated, allowing prediction of crop loss for a certain level of disease at a particular point in the crop's growth. This method is dependent upon the assumption that the treatments used to keep disease at a certain level have no effect on crop yield themselves. This might not always be the case. Similarly, assessments using susceptible and resistant cultivars rely on both cultivars having a similar yield to start with. Again, this might not be true, and must be determined fist in a disease-free environment. All aspects of experimental design need to be carefully considered in order to gain an accurate assessment of the effect of disease on crop yield. For example, harvesting methods under experimental conditions are often more efficient than under field conditions, giving the appearance of a higher crop yield. From crop loss assessment studies, models can be devised. Crop loss models are usually based on one of three types of disease assessment: disease at a critical point in development, disease at multiple points in development, and disease throughout crop development.

The statistical approach to assessing disease involves statistical analysis of crop yields under different levels of disease that occur naturally in the field. The levels of disease and the crop yields are monitored, but not manipulated, and then yields from different seasons or areas with different levels of disease can be compared to determine the effect of disease on crop yield. The advantage of this method is the use of outcomes of real cropping situations in the field, not experimentally manipulated crops. The disadvantage of this approach is that in the field, the conditions are not controlled, and there are many factors that could affect yield besides disease. An alternative statistical approach to crop loss assessment is the use of questionnaires, filled in by the farmers, that allow disease and pest incidence and severity to be related to the yields they produce.