

Review

Assessing the Consequences of Microbial Infection in Field Trials: Seen, Unseen, Beneficial, Parasitic and Pathogenic

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Abstract: Microbial infections of crop plants present an ongoing threat to agricultural production. However, in recent years, we have developed a more nuanced understanding of the ecological role of microbes and how they interact with plants. This includes an appreciation of the influence of crop physiology and environmental conditions on the expression of disease symptoms, the importance of non-pathogenic microbes on host plants and pathogens, and the capacity for plants to act as hosts for human pathogens. Alongside this we now have a variety of tools available for the identification and quantification of microbial infections on crops grown under field conditions. This review summarises some of the consequences of microbial infections in crop plants, and discusses how new and established assessment tools can be used to understand these processes. It challenges our current assumptions in yield loss relationships and offers understanding of the potential for more resilient crops.

Keywords: disease assessment; asymptomatic; yield loss; infection; stress; crops; field trials

1. Introduction

The history of crop production is largely defined by its relationship with microbial infection. Pathogenic plant microbes infecting staple crops have the capacity to cause devastating losses, with many defining events of human history being directly caused by crop disease. During the last century, the effects of crop epidemics have been diminished by reduced regional dependency on single crop species, improved crop management and chemical treatments and better global food distribution.

However, populations of plant pathogens do not remain constant and low genetic variation in elite crop varieties [1], increased global demand for food, and the effect of climate change on pathogen prevalence and distribution, provide new challenges to crop protection [2,3].

In order to meet these challenges, it is important that we are able to conduct robust and reliable field experiments to detect and quantify infection in crops under (as near as is possible) realistic agronomic conditions. Such experiments might be directed towards identifying genetic elements responsible for host resistance, examining the effect of agronomic practices or environmental conditions on disease development, quantifying disease management using pesticides, monitoring varietal performance as pathogen populations change, or determining the effect of crop disease on crop yields.

Most plant pathogens are associated with clear and characteristic visible disease symptoms. Indeed, common names for crop diseases usually reflect the visible signs of infection. These symptoms may take the form of over/under development of plant organs, necrosis of plant tissue, or alterations from normal appearance. Disease symptoms can have direct impacts on key host metabolic processes such as photosynthesis or nutrient uptake largely through loss of leaf area for plant processes e.g., [4,5]. However, infection prior to symptom expression (or even incompatible host-pathogen interactions) can have significant effects on host metabolism [6–8], whilst disease symptoms on crop structures that are not yield limiting may have little or no effect [9]. As such, it is becoming clear that relying solely on visible disease symptoms as a proxy for disease severity or to inform yield loss models will impose significant limitations on our understanding of microbial interactions with crop plants and their effects.

In addition to an increased appreciation of the effects of asymptomatic infection we also now understand that plants may act as hosts for a variety of other microbes that protect against infection, improve tolerance to abiotic stress or increase yield [10]. In crops grown for food production, the capacity of plants to serve as hosts for human pathogens must also be considered [11].

Therefore, it is clear that in recent years, advances in methods for detecting, quantifying and visualizing microbes on crop plants have led to a much clearer understanding of the complexity of microbial interactions with both host plants and other microbes. This paper will summarise some of the key findings of this area of research and examine how they impact on our understanding and practice of crop protection.

2. Visual Disease Assessment

Assessment of visible disease symptoms remains the primary method for detecting and quantifying most crop diseases, both under controlled environments and in field trials. Visible disease assessments have been routinely used for many years in order to monitor varietal performance, for mapping major and quantitative resistances to crop pathogens or assessing the impact of various crop protection practices. Whilst visual assessment provides a relatively cheap and quick method for assessing entire field plots, such measurements are not necessarily true reflections of overall levels of infection [12]. Furthermore, crops grown under field conditions can be exposed to a variety of plant pathogens, whilst the majority of major crop pathogens produce symptoms that are distinct and recognisable by appropriately trained field workers, less common pathosystems may produce similar or identical disease symptoms e.g., [13,14]. Furthermore, in some cases, closely related pathogen species may not be distinguished by symptom expression despite differences in host pathogen interactions e.g., [15] or

agricultural significance [16]. Abiotic stresses (often in conjunction with crop genotype) can produce phenotypes that are superficially similar to disease symptoms [17]. As such, assessors require a high level of training and experience to be able to recognize and distinguish between common pathogens as well as physiological, disease-like, phenotypes.

2.1. Field Scoring Visible Disease Symptoms

For systemic diseases, or diseases that invariably kill whole plants, disease incidence may be a sufficient description e.g., [18], with disease on crops or plants being reported as either present or absent. Similarly, when disease levels are low, incidence may be an adequate approximation of severity. However, in most cases it is necessary to score disease severity as a quantitative trait. Most quantitative visual disease assessment protocols use disease severity keys with descriptions of symptoms that relate to varying levels of disease severity e.g., [12]. Furthermore, heterogeneity in disease severity may exist within field plots, whilst the relationship between subjective assessment of disease and true levels of infection is not always simple [12,19]. Inevitably, this leads to large subjective components in disease ratings [20]. For a single assessor scoring a single trial this may have little significance, but when comparisons are made between trials, or multiple assessors are used for scoring single trials, these must be accounted for [21]. A recent study examining the effect of subjectivity on the ability to detect QTL for northern leaf blight resistance in maize concluded that whilst variation between scorers was unlikely to affect the estimation of QTL locations, significant variation was detected in the estimation of QTL effects [22].

A number of attempts have been made to remove subjective components in disease assessment by automating symptom identification and quantification using image analysis techniques (reviewed in [23]). Whilst such methods are now being employed in field settings e.g., [24,25], and can, in some cases, distinguish between symptoms caused by different pathogens, the applications of image analysis methods are generally too specific to be of use as a general tool [23]. However, this area of research is developing rapidly, and improvements in computational techniques and imaging technologies has the potential to offer generalised tools for studying field infection [23].

2.2. Physiological and Environmental Interactions with Symptom Expression

Whilst most of the variation in levels of symptom expression observed in crops is caused by variation in levels of infection, a number of physiological, epidemiological, or environmental factors have been demonstrated to influence expression of disease symptoms [26]. Likewise, host genotypes can have important consequences on the levels of disease symptom expression following infection [26,27].

It is well known that keeping the flag leaf “clean” in wheat is crucial for protecting yield [28]. This is because up to 40% of the assimilates going to grain-fill are produced by the flag leaf [29]. This begs the question as to how important microbial challenges are to other parts of the plant and what is the temporal dynamic? For example, early leaves that later become the lower leaves of cereals may contribute effectively to plant establishment including tiller production and grain primordial number, but contribute nothing directly to grain fill. Many tillers later abort as the growth potential is matched to resources [30]. Thus, at any given time, leaves may have very different importance and this is reflected in their ability to express resistance to pathogen challenge. Old leaves down-regulate defence

mechanisms [30] and are thus vulnerable to pathogen attack or, more likely, saprophyte colonisation. In non-crop plants this may have few consequences, however for crops with greatly extended grain filling periods it could still have some detrimental effects. Furthermore, such leaves can act as reservoirs of inoculum for infection of younger photosynthetically active leaves and thereby further damage yield whether by infection and necrosis or the costs of defence mechanism induction [31]. Developmental stage, leaf physiological state, pathogen infection and symptom expression are all pertinent parameters for assessment of field trials.

Symptom expression, whether attributable to necrosis or visible pathogen biomass such as with powdery mildews or rusts, can be assessed by eye but may not be representative of the damage caused or amount of pathogen present. Pathogen growth may be described as explorative or exploitative depending on whether it is more extensive as it searches for assimilates and nutrients or has sufficient. Such exploration or exploitation strategies may be due to resistance expression or nutrient accessibility/abundance differences [32]. They are therefore worth characterising, but thin exploratory growth may not be visible to the naked eye. Therefore more objective assessments of pathogen biomass would be useful to use in conjunction with these assessments.

3. Molecular Detection and Quantification of Crop Pathogens

It is now recognised that many common crop pathogens may have significant and complex relationships with their hosts prior to the onset of disease symptoms, and disease symptoms may not unambiguously identify the pathogen. As such, alternative methods for detecting microbial infection in crop plants have been developed, both for the identification and early detection of infection, and to investigate host pathogen interactions during the asymptomatic phase.

PCR-based molecular detection of plant microbes became available in the early 1990s [33], and offered rapid and highly specific assays. Pathogen specific PCR primers have been developed for many phytopathogens e.g., [14,34–41]. A major limitation of conventional PCR assays is their inability to quantify infection from field-collected crop samples. However, related methods have been developed to address this limitation. The most commonly used method is quantitative polymerase chain reaction (qPCR). qPCR-derived copy number estimates of pathogen specific amplicons allows the measurement of overall infection levels from total DNA extractions made from infected plant material. As absolute copy number will depend on the size of the sample, and such protocols use either a standard quantity of total DNA e.g., [36,41], or report pathogen specific amplicons relative to those of the host e.g., [34]. Such an approach also has the advantage of accounting for variation in DNA extraction efficiency between samples. Whilst this is convenient, infection is more usefully compared to the volume of plant tissue or leaf surface area. Some studies have tested for the effects of host cell death on host specific amplicons [34], but the effect of variation in host DNA copy number (per unit area or mass) due to variation in host cell size (caused by leaf age or genotype effects) has not yet been studied. Genotypic effects on cell size have been reported in a number of crop species, and significantly, some of these effects (caused by dwarfing genes) have also been shown to influence host pathogen interactions [42]. We know that resistance response to pathogen challenge is affected by cell type and size [43]. Similar effects have been proposed due to variation in hyphal cell size in fungi [44],

whilst variation in contribution to qPCR based estimates of fungal colonisation have been reported between spores and hyphae [45].

Unlike visible disease assessments, it is not feasible to make qPCR assessments of entire plots. Pathogens may show high levels of spatial heterogeneity within plants [45] and across different spatial scales [46]. Sampling strategies require careful consideration to ensure that estimates are both representative and appropriate to the question being asked. There has been much work on field assessment strategies based on visual symptoms, so typically diagonal, “X” or “W” shaped walks are used to sample fields [47]. In small plot field trials either the whole plot should be sampled randomly or on a regular grid, or only specific parts sampled. This would generally be to account for edge effects that can be a large proportion of the plot. These considerations apply to the horizontal plane but equally important is the vertical plane, complicated by plant growth stage considerations. Thus a stratified sample of different leaf layers is ideal, but constraints on sample size and number must be informed by knowledge of the epidemiology of the target microbes.

Another consequence of the restricted nature of sampling for qPCR assays, coupled with often large experimental error associated with the methodology is that quantitative traits based on such methods can lack statistical power, reflected in relatively low heritability estimates of qPCR based assessments of infection [27]. However, qPCR-derived infection scores generally correlate well with visible disease assessments [36,48] and offer the potential for early identification of infection levels when experiments are carefully planned and conducted.

qPCR disease assessments also offer the opportunity to examine the extent and consequence of asymptomatic infection when combined with symptom data. This can be achieved by specifically testing asymptomatic leaves e.g., [49,50] but a more general approach is to examine levels of symptom expression relative to total infection by accounting for the relationship between the two traits. Such an approach has been used to identify genetic loci that have differential effects on overall infection relative to symptom development [27]. This offers the opportunity to integrate studies of host resistance with advances in the understanding of the range of interactions between pathogens and their hosts [9].

An alternative molecular method of detecting plant disease during early infection is the use of immunoassays such as enzyme-linked immunosorbent assay (ELISA). Such methods are quicker and cheaper to perform than qPCR based assays and as such may be appropriate where disease incidence is of primary concern [51]. Another method is the quantification of fungal material, typically cell wall components, by chromatography. This was found to be more accurate than image analysis or counting colonies of powdery mildew [52,53]. However, the method is very time-consuming and, like qPCR, expensive. Image analysis methods to assess symptoms to provide more objectivity can be employed also on sampled leaves. However, parameter setting for analysis often has subjective components and the same statistical and sampling considerations apply to these methods as to qPCR data.

Airborne dispersal is a critical epidemiological stage for a number of important crop pathogens, and methods for detecting levels of airborne inoculum for understanding pathogen epidemiology and forecasting disease are available [54]. Spore traps to capture ascospores have been available for many years, but analysing spore traps using conventional microscopy is both time-consuming and difficult. Combining spore traps with qPCR methods has allowed a number of studies to assess the importance of such dispersal mechanisms in a number of pathosystems, with such information also being used to inform epidemiological models [55].

4. Measurements of Host Response to Stress

The use of qPCR-based methods for early pathogen detection may be limited when pathogen distribution is highly localised in host tissue, or where primary disease spread is associated with very low levels of pathogen DNA. As such, in recent years interest has been expressed in the development of biomarkers based on host defence responses. Such markers are likely to involve the identification of host genes or sets of genes that are specifically up-regulated following pathogen challenge [44,45]. This will require detailed knowledge of gene networks involved in host defence responses, but has the potential to offer not only early indication of pathogen attack, but will also offer valuable insights into the nature of the host pathogen interaction and even methods for disease management [56]. Whilst this approach may lack the specificity of PCR-based detection methods, it has the advantage of reporting detailed information about host recognition and response to pathogen challenge.

As well as specific markers based on host defence responses, damage or stress caused by infection can also offer insights into the extent and effect of infections. We noted earlier that developmental stage, leaf physiological state, pathogen infection and symptom expression are all pertinent parameters for assessment of field trials. To measure and weight all such parameters in a field trial is logistically impractical, thus any methods that integrate these effects into single, preferably automated and highly objective measurements would be very attractive. As yield is ultimately dependent on the efficiency of photosynthesis, then direct measurement of this would be desirable. Chlorophyll fluorescence imaging does this as well as being able to identify cell death before it is visible, and with higher contrast than screening under visible light. Chlorophyll fluorescence methods have been used in a variety of pathosystems [57–59], and offer the opportunity to identify the destructive effects of infection before disease symptoms become visible.

Measuring chlorophyll fluorescence is still somewhat labour-intensive and therefore subject to rationalisation with sampling strategies, compromising rapid assessment of extensive replicated field trials. A less direct method is to measure crop reflectance. This effectively integrates chlorophyll visualisation with other morphological and physiological expressions of plant health. Because it is a step removed from direct measurements it must be calibrated, but as long as suitable controls or standards are included amongst treatments it is very suitable for comparing crop protection treatments in particular [60]. They are not suitable for assessment of trials where phenotypic differences are caused by plant genotype differences as morphological trait variation may be greater than other treatment differences and thus obscure effects. Assessments were made with instruments such as the Cropscan Radiometer that was very sensitive to light angle and intensity. Later instruments with their own light source such as the “Greenseeker” instrument, recording data directly to excel files in a PDA became quicker and more user-friendly. Currently, there are either farm machinery mounted and integrated with treatment application technology, or truly hand-held instruments that are robust across a wide range of light conditions and are easy and quick to use. Such technologies are reviewed elsewhere in this volume. Yield loss correlations from such instruments are often better than the area under the disease progress curve or individual disease scores. However, it is not possible to identify in advance the cases where this is true and as such these technologies do not yet represent an alternative to traditional assessments.

5. “Non-Pathogenic” Microbe Interactions, with Pathogens, Each Other, and with Their Host

In field trials we focus on the negative impacts of microbes, classifying them mainly as pathogens when we observe symptoms. However, we normally assess only disease as discussed above. Whilst successful pathogens that result in extensive symptoms will represent the dominant microbial species, many other microbial species will be present. Some species, not normally considered pathogens in their own right, become part of the disease complex and could be considered opportunistic pathogens when they can be demonstrated to enhance disease symptoms. These will often be bacterial species that happen to be commonly present [61–63]. Which species are present is likely to be less important than the total bacterial load, and the latter will be strongly influenced by soil properties, environment/climate/weather and crop history. Their effect may be to enhance or reduce disease caused by a primary or dominant pathogen. They may induce susceptibility or resistance, but also work synergistically with pathogens to break down host tissue more rapidly [64]. They may reduce disease by competition with the pathogen, hyperparasitism or by causing disruption of pathogen infection processes. At present little work has been done to assess the impact of non-pathogenic microbes on disease expression. However, we know that high bacterial loads from, for example, a prior potato crop with severe black-leg disease (causal agent *Pectobacterium atrosepticum*) can in subsequent cereal crops enhance *Septoria tritici* blotch (STB) and powdery mildew (*Blumeria graminis* f. sp. *tritici*), whilst early season *Rhynchosporium* infection (*Rhynchosporium commune*) can be reduced [61]. Thus risk factors could be described, or disease pressure specifically enhanced for better resistance appraisal with such knowledge. Molecular techniques have revealed many more phylloplane and phyllosphere species than were hitherto detected by cultural means but too little is known about such microbial relationships yet for any particular approaches to be recommended.

A classic example of risk factor enhancement is following maize crops with cereals. Maize debris builds up inoculum of *Fusarium* species, especially *F. graminearum* [65]. An associated risk factor is minimum tillage, *i.e.*, leaving more crop debris around the stem base or emerging seedlings where saprophytic fungal growth can produce enhanced levels of inoculum able to infect the crop. Eyespot (*Oculimacula yallundae*) on wheat is an example where the debris retained in minimum tillage causes enhanced disease [66–68]. However, in some circumstances this may decline following continuous minimum tillage, thought to be due to development of more balanced microbial populations attenuating the eyespot inoculum [69,70]. In general inversion tillage reduces infection by splash-dispersed pathogens able to sporulate as saprophytes on crop debris. For *R. commune* we have demonstrated over many years that this is certainly true for early season infection levels [71] but like others [72] this was not a big factor overall for this pathogen.

Seed-borne infections may be the source of not only pathogens, but also many of the other microbial components of both the rhizosphere and phylloplane. It is known that, for example, *Rhynchosporium commune* inoculum can be transmitted through contaminated seed although its importance in subsequent epidemic development depends on epidemiological factors that vary between sites and seasons [73,74]. With *Ramularia collo-cygni* the infection is throughout both embryo and non-embryo material and spreads systemically throughout the host crop plant [75]. Clearly the level of seed transmission affects the risk to subsequent crops and the requirement and difficulty of controlling the infection [76]. It is not known whether other non-pathogens are transmitted through seed infection,

whether they have beneficial or detrimental effects, and what the consequences of pathogen control treatments might be on these microbes. There is potential for such microorganisms to be developed further as biocontrol agents. All these factors may affect field performance of subsequent crops and therefore quantitative molecular microbial profiling of crops in future may be an important component of field trial assessment and interpretation once the likely consequences of such colonisations are known.

Soil-borne diseases are another case where knowledge of cropping history is crucial to assessing risk. The classic case is take-all caused by *Gaeumanomyces graminis* var. *tritici* where second and third wheat crops suffer yield decline more serious in some cultivars than others [77]. Above-ground symptoms are only seen when the problem has become so severe that “white heads” are seen. The risk can also be associated with the previously grown cultivar, presumably attributable to differential inoculum production of different cultivars. However, the phenomenon of take-all decline is also well known where after the problem peaks it subsequently declines. Again, this phenomenon is thought to be due to the ecological balance of microbes attenuating inoculum production from the problematic pathogen [77].

Taken together, these examples illustrate the importance of understanding the ecology of not just so-called pathogens but also other microbes and how they might exacerbate or attenuate the effects on pathogen inoculum and pathogenesis.

6. End-User Consequences of Microbial Infection

Another factor not normally taken into consideration in field trial assessment is “contamination” with other organisms not necessarily causing known impacts on yield, but having implications for down-stream processing. These include mycotoxins, the most common ones of concern being DON, ZON, NIV, HT2 and T2 from infection with some *Fusarium* species [78]. However, not all *Fusarium* Head Blight (FHB) causal agents produce toxins. *Microdochium nivale* and *M. majus* can also be part of this complex, particularly in cooler humid conditions, but these produce no known mycotoxins [79]. Nor are they always directly correlated with known toxin producing species [80,81].

Several crop species are now known to not only be contaminated with, but also propagate bacterial pathogens of animals and humans such *Escherichia coli* and *Salmonella enterica* serovars as well as plant bacterial pathogens of other crops [11]. Although of more importance in some vegetable crops consumed directly, they are nevertheless also of concern for operational safety and animal feed in large acreage combinable crops. However, these are not generally the target of any breeding or crop protection programmes, so simply sampling and qPCR for detection above threshold levels in crops of concern is adequate. In vegetable crops the consequences for such potentially contaminating bacteria needs to be assessed in relation to potential impacts on crop safety. Novel resistance elicitor-based approaches targeted towards bacterial pathogens are being developed and would have particular utility on such crops as few other approaches are effective.

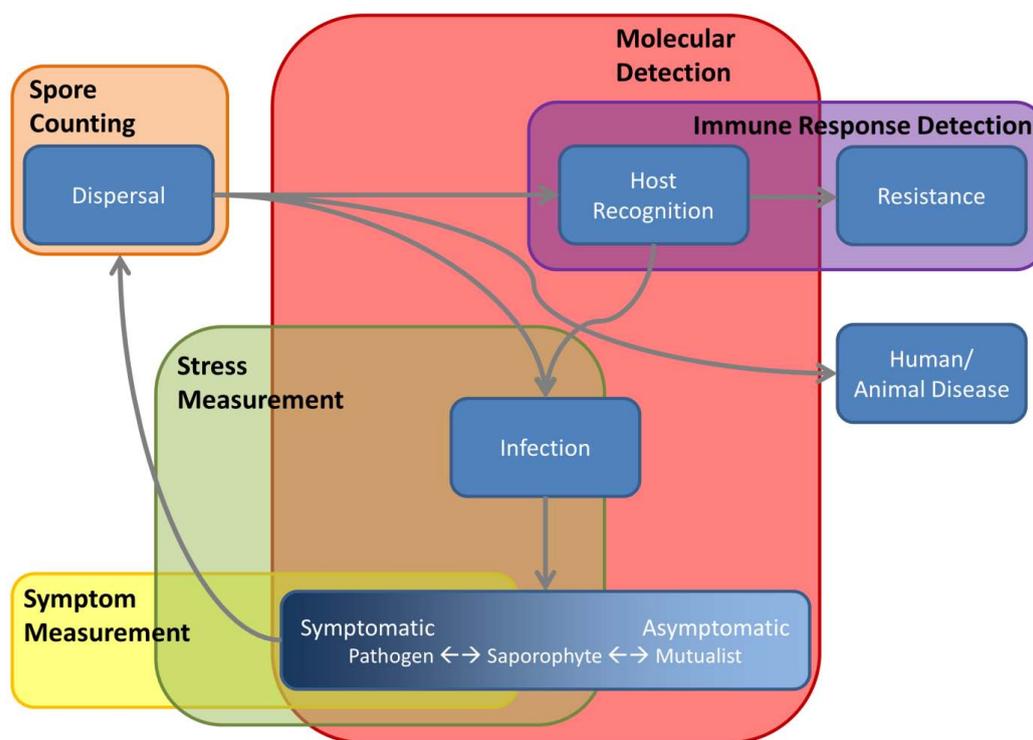
7. Yield Loss Implications of Different Microbial Infections

We discussed asymptomatic infection above in the context of its genetic control and triggers for symptom development. Whilst symptoms cause obvious loss of green leaf area and thereby reduce yield, asymptomatic infection can be very extensive and must also obtain resources from the host plant

and have the potential to cause yield loss. Newton *et al.* [9] argued that organisms such as those causing the cereal rusts and powdery mildew diseases although commonly referred to as pathogens are in fact parasites as they damage the host their host by draining resources, particularly carbon, rather than actively damaging their host by causing necrosis. In the same way, asymptomatic pathogens occupy this same niche for deriving resources from their host, *i.e.*, the same trophic space, but in their case the relationship can be dynamic. Thus triggers for symptom expression involve necrosis and thus the trophic space occupied becomes that of a pathogen. Yield loss must therefore be calibrated by assessment of microbial load and their relative impact in diverting resources to microbial biomass and/or by reducing green leaf area but the importance of these effects are no yet known.

A summary of the possible outcomes of infection in conjunction with the other factors discussed above is shown in Figure 1. Alternative methods for scoring microbial infection allow us to investigate stages of this interaction in ways that were previously unavailable.

Figure 1. Summary of major steps and outcomes associated with microbial infection of crops. Major steps in the host/microbe interaction are represented by blue boxes, with routes between these steps indicated by arrows. The phenotypic state of the infection is in the blue gradient box. Methodologies that allow investigation of these main stages are indicated.



Some microbes on or in crops can contribute beneficially to yield. Many are root-inhabiting, facilitating acquisition of specific nutrients or water in return for assimilates, mainly carbon. Mycorrhizae are classic examples of these but high soil disturbance conditions coupled with high levels of nutrients tend to mitigate against their presence and efficacy, particularly in crops such as the cereals that are not highly mycorrhizal anyway [82]. However, the response to soils and their microbial communities is seldom a factor in breeding programmes but there is much potential, particularly if fungicidal treatments are more contained, reduced or targeted [82]. This potential has been described

as the “evergreen revolution” where the beneficial relationships between crop plants and their symbionts, combined with their influence on soil chemistry, physics and biology, can be exploited as the means to achieving “sustainable intensification” [82]. However, even some fungi normally known as pathogens can have beneficial effects on plant biomass when they fail to transition to the symptomatic or pathogenic trophic state [9]. Classical yield loss relationships to crop genotype and crop protection treatments will be changed in such circumstances and therefore quantification of such endophytic symbiotic colonisations will be important to understand crop yield responses.

Disease-yield loss relationships are clear where disease levels are high [83]. Where they are low they are not only less clear but may also be confounded by plant compensation responses. For example, early damage may result in enhanced tiller production that could even result in enhanced yield, though more but smaller grains could impact quality criteria in some markets.

Determining yield loss or impact of asymptomatic infection in field trials is therefore highly problematic. Treatment with inoculum that results in high levels of pathogen challenge and possibly asymptomatic infection might be one method. This is difficult to achieve in practice but can lead to disruption of the expected relationship between symptom expression and yield loss [84]. Elimination of infection though targeted use of fungicides might be another. However, many fungicides have other effects on the crop, the triazoles and strobilurins for example, cause direct leaf greening, or a leaf greening achieved through shifts in the microbial challenges being experienced by the crop [85,86]. Using a range of modes of action along with assessment of asymptomatic infection might be a means to factor-out direct effects attributable to differential non-target effects of the fungicides. It should also not be forgotten that fungicides can shift the population structure of bacteria [87] that may affect plant recognition responses [88].

The disease level at which crop protection treatments should be applied is an area of considerable research and debate. Disease risk curves can be produced for different crops and tailored to specific cultivars showing different economic thresholds. However, the regressions often do not intercept both X (disease) and Y (probability) axes at the origin, more commonly intercepting at a Y value of a positive risk at zero disease [89], in other words some benefit of crop protection treatment is always found. This could conceivably be above the economic threshold or require only low disease scores to justify treatment. Part of the explanation may be attributable to asymptomatic infection being controlled by such treatments [90].

Whilst infections can be termed symptomatic or asymptomatic based on the presence or absence of clear visible symptoms, this distinction is, to some extent, a reflection of the ability of the assessor to detect the effects of infection. In reality, even non-destructive infections may have significant effects on important host functions. The commercial consequences of disease (measured by visible symptoms) have been extensively described, but relatively few studies have considered the consequence of asymptomatic infection on crop yield and quality.

8. The Ecology of Crop Phenotyping

Classic cultivar evaluation field trial treatments should not be considered as simply yields with and without disease. They should more correctly be regarded as contrasts between the effects of controlled and uncontrolled microbial challenges. We have increasing numbers of tools to enable us to do this,

particularly our “conventional” crop protectants that can be tailored by mode of action, dose and timing, particularly those that are systemic or eradicator, and resistance elicitors that can either prime or trigger the crop’s own defence mechanisms. Resistance elicitors can disrupt the normal relationship between recorded disease and yield loss, often achieving greater yield than would be expected from the disease levels observed [91]. Effectively they can mimic disease tolerance, probably due to non-target effects on gene expression such as up-regulation of photosynthesis.

Disease tolerance is a difficult concept to understand in field trials. Defined as the ability of a plant to produce a greater yield than would normally be expected from the observed disease [92] it can be the sum of many different components and is therefore impossible to select for in anything other than a highly defined set of parameters [93]. It may be attributable to better yield compensation mechanisms, restricted asymptomatic infections or conversely greater levels of beneficial endophyte colonization. It may be attributable to resistance priming rather than full expression in response to microbes that are not actual pathogens of that crop. Far better to understand and manipulate potential mechanisms of apparent tolerance with the tools described above than attribute tolerance traits that are likely to be environment-specific.

9. Nutrient-Agronomy-Disease: Infection Interactions

The maintenance of correct mineral nutrient levels are vital for crops to achieve their yield potential, but nutrient levels may also have significant effects on host pathogen interactions [94–96]. Certain nutrients are correlated with improved resistance to some diseases in some crops but there are few trends that cross groups of crops or diseases [64]. In susceptible cultivars, biotrophic pathogens such as the rusts or powdery mildews on cereals can be much more severe under high fertiliser inputs, particularly nitrogen e.g., [92]. This is considered to simply reflect a better supply of assimilates to what are effectively parasites and the effect is to scale the interaction rather than change it in a fundamental way. Inoculum pressure too can affect such diseases, and interact with fertiliser inputs. For example, in a barley trial, high fertilizer caused 1.77 times more powdery mildew under high inoculum conditions but 4.58 times under normal inoculum conditions [92]. In general, nutrient supply should meet, but not exceed crop demand as an imbalance or deficiency of certain nutrients can disrupt the normal effects of diseases on crops [97]. Indirect effects caused by nitrogen affecting canopy structure and thus pathogen microenvironment have also been reported [98].

Microbial infection may also influence host nutrient levels due to their effects on plant root morphology or physiology [94,95]. Perhaps of more consequence are deficiencies, particularly those that result in necrosis such as the flecking observed with manganese deficiency. There is evidence that a tendency to express such deficiencies has a genetic basis and as such, these can be used to locate QTL in the same way as disease symptoms [99,100]. Thus the ability to distinguish the difference between, for example, “physiologic flecking” and *Ramularia* symptoms can be crucial and certainly helped by qPCR methods that would give unambiguous quantification. There are also trade-offs attributable to the expression of some resistance genes. *Mlo* resistance to powdery mildew in barley is again a good example where it is associated with increased susceptibility to *Ramularia collo-cygni*, *Magnaporthe oryzae*, *Cochliobolus sativus*, *Fusarium graminearum* and *Pyrenophora teres*, although also sometimes associated with reduced susceptibility to *Rhynchosporium commune* [101].

10. Yield Effects of Crop Diseases

Whilst the severity of disease may be an important measure in its own right for many field experiments, disease is primarily a concern due to its effect on yield in crop plants. As such, a number of attempts have been made to use disease severity measurements to inform crop models. Such models generally make a distinction between radiation interception efficiency (RIE) and radiation use efficiency (RUE). The most obvious effect of foliar crop diseases is a decrease in RIE due to the loss of photosynthetically active tissue either from necrosis, early senescence, or (in severe infections) from reduction in leaf formation. The impact of infection on RIE is not generally even throughout the plant, with infection of upper leaves having a disproportionate effect. As such, the vertical position of disease within a crop must be accounted for. Similarly, infection can also reduce RUE. A proportion of this reduction can be attributed to a reduction in green leaf area due to necrosis, but it has been recognised for a number of years that reductions in photosynthetic capacity due to infection can extend beyond visibly damaged regions. Bastianns [102] introduced the concept of the virtual lesion to reflect this effect, with a single parameter (β) representing the ratio between the virtual and visible lesion size. This concept has subsequently been used to examine the physiological effects of disease symptoms in a number of crop pathosystems [103–105]. The incorporation of alternative phenotyping methodologies for crop diseases is an area that has received little attention, but it seems likely that the identification of stress responses or defence biomarkers has the potential to offer useful parameters into such growth models in conjunction with disease symptoms. Similarly, molecular methods for quantifying multiple microbial infections in field trials may allow earlier indications of likely yield costs associated with infection.

11. Conclusions

We now have a number of established and emerging technologies that allow us to detect and quantify microbial colonisation of crop plants in field experiments. This has not only allowed accurate and rapid phenotyping in difficult pathosystems, but has also allowed us to quantify infection when disease symptoms are absent. Not having to rely on a single aspect of crop infection (disease symptoms), and awareness of the effects of non-symptomatic microbial infections by pathogens and non-pathogens, has allowed us to develop a much more nuanced understanding of the complex relationship between the host plant, pathogen and the wider microbial community. This is already influencing our understanding of disease epidemiology and our approach to the management of important crop pathogens. Other technologies, currently being developed, will make it feasible to identify key host responses to pathogen attack and damage that may not be associated with disease symptoms at all stages of plant growth and development. Interpretation of these data in conjunction with classical disease assessments can bring a whole new insight into field trial analysis. New components of resistance or detection and explanation of unexpected effects of crop protection treatments can be obtained by this more comprehensive understanding of crop by environmental interactions. Hence we have tried to identify and characterise more components of the environment than are normally considered, particularly the microbial components. Crucial to all these analyses is avoiding the assumption that a so-called pathogen only ever causes disease symptoms and that only

one of those microbes associated with a particular disease are having the effect on the crop. Better to determine what proportion of the effect is due to known pathogenic organisms at particular times, and what may be explained by other interactions, known and unknown. Such knowledge will provide more accuracy when creating disease risk models, and will allow more flexibility in disease management strategies. Crucial to this process is the ability to identify and quantify microbial infection from field experiments even when they do not result in visible disease symptoms. A range of established and emerging technologies have provided this capability to field researchers.

Author Contributions

Mark Looseley contributed to the overall conception of the article, and provided expertise in field and molecular phenotyping. His major writing contribution was to sections 1, 2, 3, 4, 10 and 11. He also jointly edited the article, including the incorporation of reviewer comments. Adrian Newton contributed to the overall conception of the article, and provided expertise in pathogen ecology, and pathogen interactions with agronomic practice. His major writing contribution was to sections 5, 6, 7, 8, and 9.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Cooper, H.D.; Spillane, C.; Hodgkin, T. *Broadening the Genetic Base of Crop Production*; CABI: Wallingford, Oxfordshire, UK, 2001.
2. Chakraborty, S.; Newton, A.C. Climate change, plant diseases and food security: An overview. *Plant Pathol.* **2011**, *60*, 2–14.
3. Esquinas-Alcazar, J. Protecting crop genetic diversity for food security: Political, ethical and technical challenges. *Nat. Rev. Genet.* **2005**, *6*, 946–953.
4. Zhao, D.L.; Glynn, N.C.; Glaz, B.; Comstock, J.C.; Sood, S. Orange Rust Effects on Leaf Photosynthesis and Related Characters of Sugarcane. *Plant Dis.* **2011**, *95*, 640–647.
5. Berger, S.; Sinha, A.K.; Roitsch, T. Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. *J. Exp. Bot.* **2007**, *58*, 4019–4026.
6. Gruber, B.R.; Kruger, E.L.; McManus, P.S. Effects of Cherry Leaf Spot on Photosynthesis in Tart Cherry “Montmorency” Foliage. *Phytopathology* **2012**, *102*, 656–661.
7. Bassanezi, R.B.; Amorim, L.; Bergamin, A.; Hau, B.; Berger, R.D. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. *Plant Pathol.* **2001**, *50*, 443–452.
8. Bowden, R.L.; Rouse, D.I.; Sharkey, T.D. Mechanism of Photosynthesis Decrease by *Verticillium dahliae* in Potato. *Plant Physiol.* **1990**, *94*, 1048–1055.
9. Newton, A.C.; Fitt, B.D.L.; Atkins, S.D.; Walters, D.R.; Daniell, T.J. Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. *Trends Microbiol.* **2010**, *18*, 365–373.

10. Waller, F.; Achatz, B.; Baltruschat, H.; Fodor, J.; Becker, K.; Fischer, M.; Heier, T.; Hückelhoven, R.; Neumann, C.; von Wettstein, D.; *et al.* The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13386–13391.
11. Holden, N.; Pritchard, L.; Toth, I. Colonization outwith the colon: Plants as an alternative environmental reservoir for human pathogenic enterobacteria. *FEMS Microbiol. Rev.* **2009**, *33*, 689–703.
12. Newton, A.C.; Hackett, C.A. Subjective components of mildew assessment on spring barley. *Eur. J. Plant Pathol.* **1994**, *100*, 395–412.
13. Seifers, D.L.; Martin, T.J.; Harvey, T.L.; Fellers, J.P.; Stack, J.P.; Ryba-White, M.; Haber, S.; Krokhin, O.; Spicer, V.; Lovat, N.; *et al.* Triticum mosaic virus: A New Virus Isolated from Wheat in Kansas. *Plant Dis.* **2008**, *92*, 808–817.
14. Qu, X.S.; Wanner, L.A.; Christ, B.J. Multiplex real-time PCR (TaqMan) assay for the simultaneous detection and discrimination of potato powdery and common scab diseases and pathogens. *J. Appl. Microbiol.* **2011**, *110*, 769–777.
15. Dumalasova, V.; Bartos, P. Resistance of winter wheat cultivars to common bunt, *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kuhn. *J. Plant Dis. Prot.* **2006**, *113*, 159–163.
16. Eifler, J.; Martinelli, E.; Santonico, M.; Capuano, R.; Schild, D.; Di Natale, C. Differential detection of potentially hazardous *Fusarium* species in wheat grains by an electronic nose. *PLoS One* **2011**, *6*, e21026.
17. Christensen, N.W.; Hayes, P.M. Genetics of Chloride Deficiency Expression in Barley. *Commun. Soil Sci. Plan. Anal.* **2009**, *40*, 407–418.
18. Sundaraj, S.; Srinivasan, R.; Culbreath, A.K.; Riley, D.G.; Pappu, H.R. Host Plant Resistance Against *Tomato spotted wilt virus* in Peanut (*Arachis hypogaea*) and Its Impact on Susceptibility to the Virus, Virus Population Genetics, and Vector Feeding Behavior and Survival. *Phytopathology* **2014**, *104*, 202–210.
19. Bock, C.H.; Parker, P.E.; Cook, A.Z.; Gottwald, T.R. Characteristics of the perception of different severity measures of citrus canker and the relationships between the various symptom types. *Plant Dis.* **2008**, *92*, 927–939.
20. Postman, J.; Volk, G.; Aldwinckle, H. Standardized Plant Disease Evaluations Will Enhance Resistance Gene Discovery. *Hortscience* **2010**, *45*, 1317–1320.
21. Bock, C.H.; Cook, A.Z.; Parker, P.E.; Gottwald, T.R. Automated Image Analysis of the Severity of Foliar Citrus Canker Symptoms. *Plant Dis.* **2009**, *93*, 660–665.
22. Poland, J.A.; Nelson, R.J. In the eye of the beholder: The effect of rater variability and different rating scales on QTL mapping. *Phytopathology* **2011**, *101*, 290–298.
23. Arnal Barbedo, J.G. Digital image processing techniques for detecting, quantifying and classifying plant diseases. *SpringerPlus* **2013**, *2*, doi:10.1186/2193-1801-2-660.
24. Macedo-Cruz, A.; Pajares, G.; Santos, M.; Villegas-Romero, I. Digital image sensor-based assessment of the status of oat (*Avena sativa* L.) crops after frost damage. *Sensors* **2011**, *11*, 6015–6036.
25. Lloret, J.; Bosch, I.; Sendra, S.; Serrano, A. A wireless sensor network for vineyard monitoring that uses image processing. *Sensors* **2011**, *11*, 6165–6196.

26. Huang, Y.J.; Pirie, E.J.; Evans, N.; Delourme, R.; King, G.J.; Fitt, B.D.L. Quantitative resistance to symptomless growth of *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape). *Plant Pathol.* **2009**, *58*, 314–323.
27. Looseley, M.E.; Newton, A.C.; Atkins, S.D.; Fitt, B.D.L.; Fraaije, B.A.; Thomas, W.T.B.; Keith, R.; Macaulay, M.; Lynott, J.; Harrap, D. Genetic basis of control of *Rhynchosporium secalis* infection and symptom expression in barley. *Euphytica* **2011**, *184*, 47–56.
28. Bhathal, J.S.; Loughman, R.; Speijers, J. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *Eur. J. Plant Pathol.* **2003**, *109*, 435–443.
29. Araus, J.L.; Tapia, L. Photosynthetic Gas-Exchange Characteristics of Wheat Flag Leaf Blades and Sheaths during Grain Filling—The Case of a Spring Crop Grown under Mediterranean Climate Conditions. *Plant Physiol.* **1987**, *85*, 667–673.
30. Willey, R.W.; Holliday, R. Plant population and shading studies in barley. *J. Agric. Sci.* **1971**, *77*, 445–452.
31. Brown, J.K.M.; Rant, J.C. Fitness costs and trade-offs of disease resistance and their consequences for breeding arable crops. *Plant Pathol.* **2013**, *62*, 83–95.
32. Newton, A.; Guy, D. Exploration and Exploitation Strategies of Powdery Mildew on Barley Cultivars with Different Levels of Nutrients. *Eur. J. Plant Pathol.* **1998**, *104*, 829–833.
33. Rasmussen, O.F.; Wulff, B.S. Detection of P.s. pv. pisi using PCR. In Proceedings of the 4th International Working Group on *Pseudomonas syringae* Pathovars, Florence, Italy, 10–13 June 1991; Durbin, R.D., Surico, G., Mugnai, L., Eds.; Stamperia Granducale: Florence, Italy, 1991; pp. 369–376.
34. Wessling, R.; Panstruga, R. Rapid quantification of plant-powdery mildew interactions by qPCR and conidiospore counts. *Plant Methods* **2012**, *8*, doi:10.1186/1746-4811-8-35.
35. Silvar, C.; Diaz, J.; Merino, F. Real-Time Polymerase Chain Reaction Quantification of *Phytophthora capsici* in Different Pepper Genotypes. *Phytopathology* **2005**, *95*, 1423–1429.
36. Fountaine, J.M.; Shaw, M.W.; Napier, B.; Ward, E.; Fraaije, B.A. Application of real-time and multiplex polymerase chain reaction assays to study leaf blotch epidemics in barley. *Phytopathology* **2007**, *97*, 297–303.
37. Brouwer, M.; Lievens, B.; van Hemelrijck, W.; van den Ackerveken, G.; Cammue, B.P.A.; Thomma, B.P.H.J. Quantification of disease progression of several microbial pathogens on *Arabidopsis thaliana* using real-time fluorescence PCR. *FEMS Microbiol. Lett.* **2003**, *228*, 241–248.
38. Demeke, T.; Graafenhan, T.; Clear, R.M.; Phan, A.; Ratnayaka, I.; Chapados, J.; Patrick, S.K.; Gaba, D.; Levesque, C.A.; Seifert, K.A. Development of a specific TaqMan (R) real-time PCR assay for quantification of *Fusarium graminearum* clade 7 and comparison of fungal biomass determined by PCR with deoxynivalenol content in wheat and barley. *Int. J. Food Microbiol.* **2010**, *141*, 45–50.
39. Llorente, B.; Bravo-Almonacid, F.; Cvitanich, C.; Orłowska, E.; Torres, H.N.; Flawia, M.M.; Alonso, G.D. A quantitative real-time PCR method for in planta monitoring of *Phytophthora infestans* growth. *Lett. Appl. Microbiol.* **2010**, *51*, 603–610.

40. Zhu, Y.; Lim, S.S.; Schenck, S.; Arcinas, A.; Komor, E. RT-PCR and quantitative real-time RT-PCR detection of Sugarcane Yellow Leaf Virus (SCYLV) in symptomatic and asymptomatic plants of Hawaiian sugarcane cultivars and the correlation of SCYLV titre to yield. *Eur. J. Plant Pathol.* **2010**, *127*, 263–273.
41. Taylor, J.M.G.; Paterson, L.J.; Havis, N.D. A quantitative real-time PCR assay for the detection of *Ramularia collo-cygni* from barley (*Hordeum vulgare*). *Lett. Appl. Microbiol.* **2010**, *50*, 493–499.
42. Saville, R.J.; Gosman, N.; Burt, C.J.; Makepeace, J.; Steed, A.; Corbitt, M.; Chandler, E.; Brown, J.K.M.; Boulton, M.I.; Nicholson, P. The “Green Revolution” dwarfing genes play a role in disease resistance in *Triticum aestivum* and *Hordeum vulgare*. *J. Exp. Bot.* **2011**, doi:10.1093/jxb/err350.
43. Baker, S.J.; Newton, A.C.; Gurr, S.J. Cellular characteristics of temporary partial breakdown of mlo-resistance in barley to powdery mildew. *Physiol. Mol. Plant Pathol.* **2000**, *56*, 1–11.
44. Tellenbach, C.; Grunig, C.R.; Sieber, T.N. Suitability of quantitative real-time PCR to estimate the biomass of fungal root endophytes. *Appl. Environ. Microbiol.* **2010**, *76*, 5764–5772.
45. Gamper, H.A.; Young, J.P.W.; Jones, D.L.; Hodge, A. Real-time PCR and microscopy: Are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? *Fungal Genet. Biol.* **2008**, *45*, 581–596.
46. Skelsey, P.; Newton, A. Scale-dependent assessment of relative disease resistance to plant pathogens. *Agronomy* **2014**, *4*, 178–190.
47. Cooke, B.M. Disease assessment and yield loss. In *The Epidemiology of Plant Diseases*; Cooke, B.M., Jones, D.G., Kaye, B., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 43–80.
48. Garces, F.F.; Gutierrez, A.; Hoy, J.W. Detection and Quantification of *Xanthomonas albilineans* by qPCR and Potential Characterization of Sugarcane Resistance to Leaf Scald. *Plant Dis.* **2013**, *98*, 121–126.
49. Abd-Elsalam, K.; Bahkali, A.H.; Moslem, M.; de Wit, P.J.G.M.; Verreet, J.-A. Detection of *Mycosphaerella graminicola* in Wheat Leaves by a Microsatellite Dinucleotide Specific-Primer. *Int. J. Mol. Sci.* **2011**, *12*, 682–693.
50. Trivedi, P.; Sagaram, U.S.; Kim, J.S.; Brlansky, R.H.; Rogers, M.E.; Stelinski, L.L.; Oswald, C.; Wang, N. Quantification of viable *Candidatus Liberibacter asiaticus* in hosts using quantitative PCR with the aid of ethidium monoazide (EMA). *Eur. J. Plant Pathol.* **2009**, *124*, 553–563.
51. Wang, Z.C.; Yu, D.D.; Li, X.Y.; Zeng, M.J.; Chen, Z.; Bi, L.; Liu, J.J.; Jin, L.H.; Hu, D.Y.; Yang, S.; *et al.* The Development and Application of a Dot-ELISA Assay for Diagnosis of Southern Rice Black-Streaked Dwarf Disease in the Field. *Viruses* **2012**, *4*, 167–183.
52. Newton, A.C. Detection of components of partial resistance to mildew (*Erysiphe graminis* f. sp. *hordei*) incorporated into advanced breeding lines of barley using measurement of fungal cell wall sterol. *Plant Pathol.* **1990**, *39*, 598–602.
53. Newton, A.C. Measuring the sterol content of barley leaves infected with powdery mildew as a means of assessing partial resistance to *Erysiphe graminis* f. sp. *hordei*. *Plant Pathol.* **1989**, *38*, 534–540.
54. Fitt, B.D.L.; McCartney, H.A.; West, J.S. Dispersal of foliar plant pathogens: Mechanisms, gradients and spatial patterns. In *The Epidemiology of Plant Diseases*; Cooke, B.M., Jones, D.G., Kaye, B., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 159–192.

55. West, J.; Bravo, C.; Oberti, R.; Moshou, D.; Ramon, H.; McCartney, H.A. Detection of Fungal Diseases Optically and Pathogen Inoculum by Air Sampling. In *Precision Crop Protection—The Challenge and Use of Heterogeneity*; Oerke, E.-C., Gerhards, R., Menz, G., Sikora, R.A., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 135–149.
56. Martinelli, F.; Reagan, R.L.; Uratsu, S.L.; Phu, M.L.; Albrecht, U.; Zhao, W.; Davis, C.E.; Bowman, K.D.; Dandekar, A.M. Gene regulatory networks elucidating huanglongbing disease mechanisms. *PLoS One* **2013**, *8*, e74256.
57. Wagner, A.M.W.; Jamiokowska, A. Chlorophyll fluorescence measurements as indicators of fusariosis severity in tomato plants. *Agron. Res.* **2006**, *4*, 461–464.
58. Chaerle, L.; Hagenbeek, D.; Bruyne, E.; Straeten, D. Chlorophyll fluorescence imaging for disease-resistance screening of sugar beet. *Plant Cell Tissue Org. Cult.* **2007**, *91*, 97–106.
59. Pineda, M.; Olejnickova, J.; Csefalvay, L.; Baron, M. Tracking viral movement in plants by means of chlorophyll fluorescence imaging. *J. Plant Physiol.* **2011**, *168*, 2035–2040.
60. Newton, A.C.; Hackett, C.A.; Lowe, R.; Wale, S.J. Relationship between canopy reflectance and yield loss due to disease in barley. *Ann. Appl. Biol.* **2004**, *145*, 95–106.
61. Newton, A.C.; Toth, I.K. Helper bacteria and pathogenicity assessments. *New Phytol.* **1999**, *144*, 385–386.
62. Newton, A.C.; Toth, I.K.; Neave, P.; Hyman, L.J. Bacterial inoculum from a previous crop affects fungal disease development on subsequent nonhost crops. *New Phytol.* **2004**, *163*, 133–138.
63. Darling, D.; Harling, R.; Simpson, R.A.; McRoberts, N.; Hunter, E.A. Susceptibility of Broccoli Cultivars to Bacterial Head Rot: *In Vitro* Screening and the Role of Head Morphology in Resistance. *Eur. J. Plant Pathol.* **2000**, *106*, 11–17.
64. Dewey, F.M.; Wong, Y.L.; Seery, R.; Hollins, T.W.; Gurr, S.J. Bacteria associated with *Stagonospora* (*Septoria*) *nodorum* increase pathogenicity of the fungus. *New Phytol.* **1999**, *144*, 489–497.
65. Goswami, R.S.; Kistler, H.C. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* **2004**, *5*, 515–525.
66. Brooks, D.H.; Dawson, M.G. Influence of direct-drilling of winter wheat on incidence of take-all and eyespot. *Ann. Appl. Biol.* **1968**, *61*, 57–64.
67. Yarham, D.J.; Norton, J. Effects of cultivation methods on disease. In *Strategies for the Control of Cereal Disease*; Jenkyn, J.F., Plumb, R.T., Eds.; Blackwell Scientific Publications: Oxford, UK, 1981.
68. Burnett, F.J.; Hughes, G. *The Development of a Risk Assessment Method to Identify Wheat Crops at Risk from Eyespot*; Home-Grown Cereals Authority: London, UK, 2004.
69. Jalaluddin, M.; Jenkyn, J.F. Effects of wheat crop debris on the sporulation and survival of *Pseudocercospora herpotrichoides*. *Plant Pathol.* **1996**, *45*, 1052–1064.
70. Kuntzsch, E. Yield, yield components and infection with *Pseudocercospora herpotrichoides* during long-term winter wheat monoculture in the Etdorf teaching and research station 1972–1987. *Wiss. Z. Martin-Luther-Univ. Halle-Wittenb. Math.-Naturwiss. Reihe* **1990**, *39*, 107–113.
71. Newton, A.C.; Guy, D.C.; Bengough, A.G.; Gordon, D.C.; McKenzie, B.M.; Sun, B.; Valentine, T.A.; Hallett, P.D. Soil tillage effects on the efficacy of cultivars and their mixtures in winter barley. *Field Crops Res.* **2012**, *128*, 91–100.

72. Turkington, T.K.; Clayton, G.W.; Klein-Gebbinck, H.W.; Lupwayi, N.Z.; Harker, K.N.; O'Donovan, J.T.; Burnett, P.A.; Xi, K. Impact of crop management on leaf diseases in Alberta barley fields, 1995–1997. *Can. J. Plant Pathol.* **2006**, *28*, 441–449.
73. Fountaine, J.M.; Shaw, M.W.; Ward, E.; Fraaije, B.A. The role of seeds and airborne inoculum in the initiation of leaf blotch (*Rhynchosporium secalis*) epidemics in winter barley. *Plant Pathol.* **2010**, *59*, 330–337.
74. Oxley, S.J.P.; Havis, N.D.; Burnett, F.J.; Roberts, A.M.I. Spread and early control of *Rhynchosporium secalis*. In Proceedings of the Dundee Conference—Crop Protection in Northern Britain 2008, Dundee, UK, 26–27 February 2008; pp. 133–138.
75. Havis, N.D.; Nyman, M.; Oxley, S.J.P. Evidence for seed transmission and symptomless growth of *Ramularia collo-cygni* in barley (*Hordeum vulgare*). *Plant Pathol.* **2013**, doi:10.1111/ppa.12162.
76. Havis, N.D.; Nyman, M.; Oxley, S.J.P. Potential of seed treatment to control *Ramularia collo-cygni* in barley. In Proceedings of the Dundee Conference—Crop Protection in Northern Britain 2010, Dundee, UK, 23–24 February 2010.
77. Hornby, D.; Bateman, G.L. *Take-All Disease of Cereals: A Regional Perspective*; CAB International: Wallingford, Oxfordshire, UK, 1998.
78. Edwards, S. *Improving Risk Assessment to Minimise Fusarium Mycotoxins in Harvested Wheat Grain*; Home Grown Cereals Authority: London, UK, 2011.
79. Edwards, S. *Investigation of Fusarium Mycotoxins in UK Barley and Oat Production*; Home Grown Cereals Authority: Caledonia House, London, UK, 2007.
80. Kriss, A.B.; Paul, P.A.; Xu, X.; Nicholson, P.; Doohan, F.M.; Hornok, L.; Rietini, A.; Edwards, S.G.; Madden, L.V. Quantification of the relationship between the environment and Fusarium head blight, Fusarium pathogen density, and mycotoxins in winter wheat in Europe. *Eur. J. Plant Pathol.* **2012**, *133*, 975–993.
81. Landschoot, S.; Waegeman, W.; Audenaert, K.; Vandepitte, J.; Haesaert, G.; de Baets, B. Toward a Reliable Evaluation of Forecasting Systems for Plant Diseases: A Case Study Using Fusarium Head Blight of Wheat. *Plant Dis.* **2012**, *96*, 889–896.
82. Bennett, A.E.; Daniell, T.J.; White, P.J. Benefits of Breeding Crops for Yield Response to Soil Organisms. In *Molecular Microbial Ecology of the Rhizosphere*; de Bruijn, F.J., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2013; pp. 17–27.
83. Savary, S.; Teng, P.S.; Willocquet, L.; Nutter, F.W., Jr. Quantification and modeling of crop losses: A review of purposes. *Ann. Rev. Phytopathol.* **2006**, *44*, 89–112.
84. Newton, A.C.; Guy, D.C.; Nadziak, J.; Gacek, E.S. The Effect of Inoculum Pressure, Germplasm Selection and Environment on Spring Barley Cultivar Mixtures Efficacy. *Euphytica* **2002**, *125*, 325–335.
85. Ruske, R.E.; Gooding, M.J.; Jones, S.A. The effects of adding picoxystrobin, azoxystrobin and nitrogen to a triazole programme on disease control, flag leaf senescence, yield and grain quality of winter wheat. *Crop Prot.* **2003**, *22*, 975–987.
86. Beck, C.; Oerke, E.C.; Dehne, H.W. Impact of strobilurins on physiology and yield formation of wheat. *Meded. Rijksuniv. Gent Fak. Landbouwk. Toegep. Biol. Wet.* **2002**, *67*, 181–187.

87. Gu, L.K.; Bai, Z.H.; Jin, B.; Hu, Q.; Wang, H.L.; Zhuang, G.Q.; Zhang, H.X. Assessing the impact of fungicide enostroburin application on bacterial community in wheat phyllosphere. *J. Environ. Sci. China* **2010**, *22*, 134–141.
88. Fountaine, J.M.; Gravouil, C.; Daniell, T.; Harling, R.; Shepherd, T.; Taylor, J.; Dickinson, M.; Newton, A.C. Leaf wax and cultivar effects on phylloplane organisms and disease in barley. *Aspects Appl. Biol.* **2009**, *98*, 207–212.
89. Hughes, G.; Burnett, F.J.; Havis, N.D. Disease risk curves. *Phytopathology* **2013**, *103*, 1108–1114.
90. Walters, D.R.; McRoberts, N.; Fitt, B.D.L. Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biol. Rev.* **2008**, *83*, 79–102.
91. Walters, D.; Walsh, D.; Newton, A.; Lyon, G. Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology* **2005**, *95*, 1368–1373.
92. Newton, A.; Guy, D.; Gaunt, R.; Thomas, W. The effect of powdery mildew inoculum pressure and fertilizer levels on disease tolerance in spring barley. *Z. für Pflanzenkrankheit. und Pflanzenschutz* **2000**, *107*, 67–73.
93. Bingham, I.; Newton, A. Crop Tolerance of Foliar Pathogens: Possible Mechanisms and Potential for Exploitation. In *Disease Control in Crops: Biological and Environmentally-Friendly Approaches*; Walters, D., Ed.; Wiley-Blackwell: Chichester, UK, 2009; pp. 142–161.
94. Dordas, C. Role of nutrients in controlling plant diseases in sustainable agriculture: A review. *Agron. Sustain. Dev.* **2008**, *28*, 33–46.
95. De la Fuente, L.; Parker, J.K.; Oliver, J.E.; Granger, S.; Brannen, P.M.; van Santen, E.; Cobine, P.A. The Bacterial Pathogen *Xylella fastidiosa* Affects the Leaf Ionome of Plant Hosts during Infection. *PLoS One* **2013**, *8*, e62945.
96. Walters, D.R.; Bingham, I.J. Influence of nutrition on disease development caused by fungal pathogens: Implications for plant disease control. *Ann. Appl. Biol.* **2007**, *151*, 307–324.
97. Silvia, H.; Elke, B.; Ewald, S. Plant disease control by nutrient management: sulphur. In *Disease Control in Crops: Biological and Environmentally-Friendly Approaches*; Walters, D., Ed.; Wiley-Blackwell: Chichester, UK, 2009; pp.221–236
98. Lemmens, M.; Buerstmayr, H.; Krska, R.; Schuhmacher, R.; Grausgruber, H.; Ruckenbauer, P. The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains. *Eur. J. Plant Pathol.* **2004**, *110*, 299–308.
99. Newton, A.; Thomas, W.T.B. Resistance to spots and blotches in spring barley. In Proceedings of the Dundee Conference—Crop Protection in Northern Britain 2006, Dundee, UK, 28 February–1 March 2006; pp. 191–194.
100. Thomas, W.T.B.; Baird, E.; Fuller, J.D.; Lawrence, P.; Young, G.R.; Russell, J.; Ramsay, L.; Waugh, R.; Powell, W. Identification of a QTL decreasing yield in barley linked to Mlo powdery mildew resistance. *Mol. Breed.* **1998**, *4*, 381–393.
101. McGrann, G.R.D.; Stavrinides, A.; Russell, J.; Corbitt, M.M.; Booth, A.; Chartrain, L.; Thomas, W.T.B.; Brown, J.K.M. A trade off between mlo resistance to powdery mildew and increased susceptibility of barley to a newly important disease, *Ramularia* leaf spot. *J. Exp. Bot.* **2014**, *65*, 1025–1037.

102. Bastiaans, L. Ratio between Virtual and Visual Lesion Size as a Measure to Describe Reduction in Leaf Photosynthesis of Rice Due to Leaf Blast. *Phytopathology* **1991**, *81*, 611–615.
103. Roloff, I.; Scherm, H.; van Iersel, M.W. Photosynthesis of blueberry leaves as affected by septoria leaf spot and abiotic leaf damage. *Plant Dis.* **2004**, *88*, 397–401.
104. Elings, A.; Rossing, W.A.; van der Werf, W. Virtual Lesion Extension: A Measure to Quantify the Effects of Bacterial Blight on Rice Leaf CO₂ Exchange. *Phytopathology* **1999**, *89*, 789–795.
105. Erickson, J.E.; Stanosz, G.R.; Kruger, E.L. Photosynthetic consequences of *Marssonina* leaf spot differ between two poplar hybrids. *New Phytol.* **2004**, *161*, 577–583.

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