# Research Review

# Variation in aggressiveness in *Phytophthora infestans*

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# 1. Grower summary

*Including practical implications of changes in aggressiveness for the GB potato industry*

## *What is aggressiveness?*

Aggressiveness is a specific trait of the late blight pathogen (*Phytophthora infestans*) related to the amount of disease caused in a given set of circumstances (e.g. crop outbreak). It is often discussed in relative terms; that is the aggressiveness of one type of blight (i.e. pathogen population) is compared to another. It is a key component of pathogen fitness which governs the longer-term 'success' of each particular type of blight.

Pathogen fitness relates to populations and is a measure of how much a given *P. infestans* genotype contributes to the gene pool of the next generation. Fitness can refer to cycles of disease on the foliage in a single crop and/or the longer-term survival from one season to the next. Aggressiveness and fitness are strongly related, but the relationship is not necessarily straightforward; in fact is likely to be very complex. In the UK, *P. infestans* relies on its potato host for survival over winter and a highly aggressive genotype of the pathogen may therefore die out if all infected tubers rot rather than sprout to form infected plants (i.e. fitness would be low). Not all increases in disease severity are related to increased aggressiveness. For example, a fungicide resistant strain may result in more disease, but the strain itself is **not** necessarily more aggressive. Aggressiveness can only be tested by comparing it to other isolates in the absence of the fungicide.

## *How is aggressiveness measured?*

Since aggressiveness is a function of the interaction of pathogen and host, and may be influenced by many other factors, it has proved very challenging to quantify in an objective and reproducible manner. Several components are generally scored; such as the percentage of spores that result in disease (infection frequency), the time between infection and spore production (latent period) and the numbers and duration of spore release (sporulation capacity). Ideally, for each series of isolates, all components should be measured against a range of relevant cultivars under carefully monitored conditions. Depending on the scale of the study, experiments can either be made on detached leaves or whole plants under controlled conditions, in growth rooms or outdoors under field conditions. There are pros and cons of each method. Recent developments in *P. infestans* genotyping mean that the simultaneous estimation of multiple isolates' aggressiveness within a single field epidemic can now be measured. A major complication in all such studies is that aggressiveness tends to be lost when a pathogen is continuously cultured in the laboratory. Even repeatedly inoculating it onto its host cannot be guaranteed to restore its original aggressiveness. This makes it difficult to compare old strains with more recently isolated ones.

 

## *Does aggressiveness vary and by how much?*

In most pathogen populations isolates differ in aggressiveness, and those of *P. infestans* are no exception. Differences in aggressiveness have been observed over a range of time and space. Consistent, differences have even been reported amongst single-spore isolates derived from a single parent isolate that are expected to be genetically uniform. Such differences however, tend to be smaller than those between genetically distinct populations. Differences in aggressiveness have resulted in the displacement of a less aggressive population with that of a more aggressive one. Variation for aggressiveness to foliage and tubers has been demonstrated, but a direct relationship is not evident, and in both cases an interaction with cultivar and environmental conditions is apparent.

## *How does aggressiveness increase?*

The relationship between aggressiveness and fitness is complex but both are under strong selection pressure that drives the long and short-term success of particular *P. infestans* populations. Both general increases in aggressiveness and cultivar-specific adaptations have been reported. The latter case implies that continual cultivation of the same variety will result in erosion of resistance but, in practice, there is little evidence for this in the field. The mechanisms and processes controlling aggressiveness are poorly understood. Although one study demonstrated that aggressiveness to tomato was simply inherited, it is more commonly believed to be a quantitative trait influenced by a complex of many different genes.

Across this range of genes two factors must be considered; mutation that changes the DNA sequence and thus the type of gene product (i.e. the genotype) and differences in the expression of existing genes in the absence of mutation (i.e. the phenotype). Changes in gene expression rather than mutation would explain published examples of rapid and reversible adaptation. Sexual recombination as a result of the occurrence of both A1 and A2 mating types will generate more variation and therefore a greater range of aggressiveness upon which selection can then act.

## *What do we know about the aggressiveness of GB* P. infestans *populations?*

There have been very few direct studies, but much can be inferred from population changes or studies in other countries. The aggressiveness of the original 19$^{th}$ century population(s) had apparently declined since it was displaced by the new population that arrived in the 1980s. Although both mating types were found in the new population, the A2, for unknown reasons, has until recently, remained at a low frequency in Great Britain. The only comparisons of GB A1 and A2 isolates (collected between 1981 and 1995) provides no support for the idea that the low A2 frequency can be explained by low aggressiveness. However, relatively few isolates were available for comparison. Recent dramatic increases in A2 incidence since 2004 seem likely to be related to increased aggressiveness but no detailed studies have been conducted to date.

 

## *Is the A2 mating type more aggressive?*

This is a common misconception. While research has shown that A2 lineages in North America, such as US-8, are aggressive, the association is simply coincidental. Many studies have confirmed a lack of association between mating type and aggressiveness. The generation of more aggressive types when A1 and A2 types mate is however a real threat. There is evidence that a new aggressive clonal lineage was generated in the US by mating of two other lineages in the field.

## *What would increased aggressiveness mean to growers?*

A straightforward consequence is more severe blight that is more costly, and perhaps environmentally damaging, to control. In addition, the number of outbreaks is likely to be greater. Spread from primary inoculum sources would have more impact, thus diminishing the success of the BPC's Fight against Blight campaign to minimise these sources. If the increase in aggressiveness was over a short time scale, e.g. 1 to 5 years, then growers would be forced to use more fungicide to control the disease. Fungicide spray intervals would have to be shortened and/or the more effective fungicides already on the market would be required. These are generally more expensive and would therefore increase growing costs. Alternatively, improved control could be achieved through the use of Decision Support Systems, e.g. Plant Plus. This should allow better targeting of fungicide inputs in relation to risk periods and therefore give a better return on the investment in fungicides. However, such systems would add to growing costs (the cost of the system plus increased management time).

If the increase in aggressiveness was more gradual, then the industry would have more control options. At present, the GB potato industry is under-utilising cultivar resistance. If a marked increase in aggressiveness is observed then the current situation, in which blight resistance rating is a low priority in cultivar selection, may have to change. The agrochemical industry continues to introduce more effective and environmentally benign fungicides but whether such incremental improvements in efficacy are sufficient to cope with any marked increase in aggressiveness is unknown. Such an increase in aggressiveness is likely to require greater integration of control measures, i.e. a combination of cultivar resistance and fungicides mediated through decision support systems based on criteria more sophisticated than Smith Periods. In The Netherlands, where increased aggressiveness has been recorded, decision support systems are being improved to take account of the fungicide requirements of cultivars with different resistance ratings (Wander *et al.*, 2006).

Decision Support System programmes and Smith Period criteria may need updating to ensure spray warnings remain timely and relevant. Clearly the consequences of any increase in aggressiveness will depend on the specific detail of the change, for example whether increases in tuber infection occur and how the interactions with specific cultivar resistance and environmental conditions evolve. If specific interactions with cultivars are observed it may influence the reliability of National List blight resistance ratings. Breeders would need to ensure they are keeping up to date and using a mix of representative aggressive strains (that also have the necessary virulence factors) for their germplasm screening and cultivar resistance assessments.

 

# 2. Introduction, terminology and scope of review

Late blight, caused by *Phytophthora infestans*, remains a great constraint to potato production in northern Europe and specifically the UK, requiring frequent fungicide applications for its control. During most of the 20th century until the late 1970s, the *P. infestans* population outside Mexico was exclusively A1 mating type and apparently consisted of a single clone. However, in the 1970s, new strains of the pathogen appeared in Europe, probably introduced from Mexico. The first sign of this was when isolates of the A2 mating type were identified in Europe, but subsequently allozyme and then molecular markers showed that what had happened was not just the introduction of some new A2 strains, but something much more radical. Spielman *et al.* (1991) were the first to propose that the old population, predominant before the 1970s, had been rapidly displaced by a newly migrating population, which was assumed to be in some way 'fitter'. The new European population comprised both new A1 and new A2 strains; by the end of the 20th century scarcely any relics of the old A1 population remained. The rapidity and completeness of this displacement and the greater problems in controlling late blight in northern Europe since the 1980s, have led to repeated claims that the new population is more aggressive than the old one. Fry *et al.* (1993) first commented that the increased fitness noted by Spielman *et al.* (1991) might be associated with increased aggressiveness. Separate migrations of *P. infestans* from Mexico (Goodwin *et al*., 1994a) introduced new populations to the US and Canada during the 1980s (Fry *et al.*, 1993), which displaced the old clonal population (Goodwin *et al*., 1994b) and were therefore assumed to be fitter. Subsequent migrations have occurred introducing new populations to many other potato-growing regions of the world (e.g. Goodwin *et al.*, 1994b; Koh *et al.*, 1994; Deahl *et al.,* 2003). Most of these were probably secondary, involving movement of the new strains from Europe and from the US and Canada as a result of international trade in potato tubers for ware or seed. However, evidence to support the hypothesis of displacement due to increased aggressiveness has only been gathered more recently and there has been no comprehensive review of the subject.

There are many clearly defined attributes of a pathogen that influence the severity of the disease it causes on a range of timescales. These attributes are often complex and inter-related and even amongst plant pathologists there has been much debate on the appropriate terminology (see Shaner *et al*., 1992; Andrivon, 1993). It is thus important to start by defining the various terms used in order to avoid confusion before considering the evidence for changes in aggressiveness.

## *Pathogenicity*

Pathogenicity relates to the ability of one organism (the pathogen) to cause disease on another (the host). Clearly *P. infestans* is able to infect and cause damage to potato and a range of other solanaceous plant species and is thus pathogenic to these crops but not to others such as cereals or oilseed rape. Subsequent terms define specific elements of pathogenicity further.

 

## *Virulence*

This is the ability of a pathogen to infect a particular host genotype (Vanderplank, 1984); it is all-or-nothing (either virulent or avirulent). In the case of *P. infestans,* virulence is used to refer to the ability of the particular *P. infestans* isolate to overcome specific R-genes, the major genes conferring resistance. There are many R-genes known, 11 of which were bred separately into 11 clones; these have been used to define the virulence/avirulence of isolates or populations of the pathogen. Some commonly grown potato varieties carry one or more of these e.g. Pentland Dell has R1, R2 and R3 and any *P. infestans* isolate which infects Pentland Dell must therefore have virulence to R1,2,3. Breeding for resistance relying exclusively on R-genes was abandoned in the 1960s, since the pathogen quickly evolved virulence to overcome them, rendering the varieties susceptible. However, clones carrying R5, R8 and R9 are rarely, if ever, overcome by European populations of *P. infestans*. The majority of potato cultivars widely-grown in the UK carry no R-genes, so knowing whether *P. infestans* isolates carry virulence to particular R-genes is not of direct practical value to growers. There is some evidence that *P. infestans* isolates from the new population carry more virulences than those from the old population, but the full set of 11 R-genes was only reported in 1969 and many older studies used only a restricted set of R1-4. Virulence to R-genes is not related to aggressiveness, which is what concerns us here. For example, several isolates of *P. infestans* may all be able to infect the potato cultivar Kennebec, which has R1, so they are all virulent on R1, but they may differ in the amount of infection that they produce on Kennebec; they are all virulent, but differ in their aggressiveness. It is aggressiveness which is the subject of this review.

## *Aggressiveness*

According to Andrivon (1993), "most plant pathologists currently use this term [aggressiveness], as originally defined by Vanderplank (Vanderplank, 1963, 1984), to designate the quantity of disease induced by a pathogenic strain on a susceptible host". The aggressiveness of a pathogen is a quantitative measure of its ability to attack a particular host and depends on the host's partial resistance. Many factors may influence a pathogen's aggressiveness and these will be discussed later. There are many variations on the definition of aggressiveness and its replacement by the term 'parasitic fitness' has been suggested (see review by Shaner *et al.,* 1992): however in this review, the term will be used as defined above by Andrivon (1993).

Andrivon (1993) also commented that one of the attributes of aggressiveness is that it can be measured under standard conditions and is then a stable trait for a particular combination of pathogen and host. In the case of *P. infestans,* the situation is complicated as the pathogen may infect both potato foliage and tubers. Since the measured aggressiveness depends on the potato cultivar as well as the pathogen and within a cultivar foliar and tuber resistance are not necessarily correlated, it is necessary to measure aggressiveness to foliage and to tubers separately.

 

## Fitness

In contrast to aggressiveness, the pathogen's fitness is the expected contribution of a phenotype to the subsequent generation (Antonovics & Alexander, 1989), and is environmentally dependent and difficult to measure. Plant pathologists have therefore generally relied on single-generation measurements of 'fitness components' (Antonovics & Alexander, 1989); fitness components such as infection frequency and sporulation are associated with aggressiveness (Day & Shattock, 1997). Despite the difficulties of measurement, it is overall fitness which will ultimately dictate which pathogen genotypes persist within the population and cause disease over time. For late blight, assessment of fitness needs to consider not just the behaviour of the pathogen in its foliar epidemic phase but its ability to survive over the winter and initiate foliar infection in the following season. This has primarily involved the study of the tuber-borne phase of the disease but, given the changes in mating type frequency, the ability of the prevalent A1 and A2 strains to generate viable oospores that are able to infect subsequent crops is an increasingly important factor.

## Interactions between these factors and how we will approach them in the review.

The terms used above are interrelated and when discussing *P. infestans* populations we need to consider the findings in the context of short-term aggressiveness and longer term parasitic fitness (e.g. Flier & Turkensteen, 1999). Aggressiveness is an important component of epidemiological success but short-term success and longer-term parasitic fitness are not necessarily simply related. An isolate that is aggressive on foliage will produce more inoculum within a crop than one of reduced aggressiveness and this may increase its chances of infecting tubers, and thus of survival to the next cropping season, i.e. its fitness. However it may also be the case that a less aggressive isolate will go unnoticed and result in a 'slow-blight' in the lower canopy that provides more opportunities to infect tubers. The fitness of such less aggressive types may also be higher as fewer seed tubers rot completely during storage with the result that more primary inoculum is carried through into the following season. Over-wintering of the inoculum in the absence of oospores is, as mentioned, a critical factor in blight epidemiology and thus the behaviour of pathogen genotypes in tubers is important. What is less clear is the interaction between aggressiveness and reproductive fitness in UK populations. We do not know if the current population contains highly aggressive A1 and A2 strains that can also mate (i.e. are compatible) and can generate abundant, viable and pathogenic oospores. This is obviously crucial both to the risk of soil-borne inoculum and the generation of new genotypes. In terms of the success of one population of blight over another, it is the relative levels of aggressiveness and fitness that are important. Fitness strongly relates to the pathogen's aggressiveness but must be seen in relation to that of other strains with which it is competing. It is thus difficult to consider these traits in isolation. Plant pathologists are, of course, concerned with factors that make disease more difficult to control and throughout this review we will consider aspects of pathogen aggressiveness that impact on the pathogen population and the success of management practices.

 

## *What will not be covered in the review*

There is often confusion between fungicide resistance and aggressiveness, particularly in the case of *P. infestans* where the first detection of resistance to the phenylamide fungicide metalaxyl in 1980 coincided with the introduction of the new population of *P. infestans* to Europe. This led to the idea that the two events were related, but they were probably coincidental. As mentioned above, aggressiveness relates to how much disease a pathogen strain causes in the absence of fungicide treatment and is unrelated to whether or not it is fungicide-resistant. A phenylamide-resistant strain of *P. infestans* will cause more disease than a -sensitive one in a crop treated with a phenylamide alone, not because it is more aggressive, but because the sensitive one is killed by the fungicide. The question of whether phenylamide-resistant strains of *P. infestans* are as aggressive as sensitive ones in the absence of a phenylamide treatment will be discussed briefly in this review, but the occurrence of fungicide resistance *per se* will not be covered.

Short- and long-term changes in climate will impact on which strains of pathogens prove most aggressive via their ability to grow and sporulate under particular environmental conditions. However, a consideration of this area in full is beyond the scope of this review.

 

# 3. Methodology

Aggressiveness has been measured in many different ways, usually reflecting the objective of the exercise and the funding available. Laboratory methods have been used when larger samples of isolates are needed, for example in studies to examine variation amongst or between populations of the pathogen. Field studies have been employed when only a few, usually native isolates were compared.

If the objective is to examine different isolates abilities to cause foliage and/or tuber disease in commercial situations, it is best to mimic the field situation as closely as possible and to determine the rate at which an isolate, introduced as a point source of inoculum, colonises foliage, sporulates, disperses, and recolonises the host, eventually to produce a primary focus of infection. Related to this is the ability of spores washed down from infected foliage to cause tuber infection leading to rapid death of the tuber, to latent infection followed by death, or to infection of stems of plants grown from the tuber as a crop or as volunteer plants in the following season.

In practice, simpler methods of assessment have usually been adopted due to funding and time constraints. What follows is a critique of the methods that have been used in published studies.

## *Laboratory methods*

Colonisation of potted plants in a growth room, or of detached leaves/leaflets/leaf discs in an illuminated incubator, allows aggressiveness, as it affects a single cycle of infection, from infection to sporulation, to be studied. These methods have the advantage that factors influencing colonisation (e.g. temperature, humidity) can be accurately controlled at levels thought to be optimal or at levels chosen to be more extreme. These are the only methods available for population studies and for those including non-native isolates.

## *Using pure cultures*

Most laboratory studies have used isolates cultured on nutrient agar and stored for variable periods at room temperature or in liquid nitrogen. Although there is anecdotal evidence that *in vitro* culture reduces aggressiveness, few published data are available (Hussain, 2003). Although most workers take the precaution of passaging isolates through leaves or tubers for one to several cycles, there is no guarantee that such treatments can restore lost aggressiveness particularly if the loss is due to genetic deletion.

Inoculation of leaf tissue is usually done by harvesting sporangia from sporulating lesions on detached leaflets or from tuber slices, counting the spores and adjusting numbers to around $10^4$ $ml^{-1}$. Droplets in the range of 10 – 50 µl are usually placed on the abaxial surface of each replicate leaflet, or whole plants are sprayed to runoff. Incubation is at 100% relative humidity (RH) within a moist chamber or in a growth room and at a temperature of 15 – 20°C with artificial light.

 

In most studies, sporangia are used as inoculum although it is generally agreed that infection in the field results largely from zoospores that are released from sporangia, encyst and form germ tubes and appressoria. If the proportion of sporangia able to form zoospores is low, due to laboratory culture, this should be taken into account. Using equal numbers of zoospores in inocula rather than equal numbers of sporangia of the isolates being compared would be preferable although handling zoospores is difficult because of the secretion of an adhesive layer during encystment.

## *Parameters assessed*

The proportion of inocula generating a sporulating lesion (**Efficiency of Infection or Infection Efficiency or Infection Frequency**) is usually scored after several days. While this gives a crude measure of the ability of several hundred spores in a droplet (typically) to penetrate and infect the host, it is not clear how it relates to the field situation where only one or a few spores land on leaf tissue and some of these initiate infection. Dyakov *et al.* (2000) used a refined technique in which 1000 spores are sprayed onto a layer of leaflets placed on a covered tray. A count of the resultant microcolonies after 2 -3 days gives a more precise estimate of infection frequency which could be compared with the percentage germination of sporangia or zoospores *in vitro*.

The period between inoculation and sporulation is known as the **Latent Period**. It has usually been measured as the mean time for the appearance of the first spores or more rarely as the time taken for 10% of the sporulating lesions to become apparent (Flier & Turkensteen, 1999).

The **rate of growth of the lesion** is commonly estimated by determining lesion diameter or area at a fixed time from inoculation or several times during growth. Sometimes the outline of the lesion is not well defined and colonisation can occur beyond the visible edge of the lesion. **The Area Under Lesion Expansion Curve** (AULEC) can be calculated as a convenient cumulative total of sporulating lesion areas (Miller *et al.*, 1998).

**Sporulation** can be measured as average number of spores produced per lesion over a defined period or can be converted to a **Sporulation Density** when expressed per unit of lesion area. Again, these are simplified measures as the periodic production and dispersal of spores over the life of a foliar or stem lesion in the field is not easy to simulate in the laboratory. Also, one can envisage *P. infestans* having evolved different ways of exploiting plant biomass to produce spores; more biotrophic forms will produce a small lesion with dense spores, whereas more necrotrophic types may yield larger lesions with spores at a lesser density but in equal number. These isolates might produce equivalent disease and thus measurements should allow the detection of such scenarios.

Some workers advocate combining some or all of the above measures into a single **Aggressiveness** or **Fitness Index** to allow ranking of many isolates. Others have argued against such indices for two main reasons; firstly, an equal weighting of all components may not be appropriate (Lebreton *et al.*, 1999) and secondly, if all components are closely correlated there is nothing to be gained in combining them (Carlisle *et al.*, 2002). Indeed, identical indices might obscure real differences in the individual components of aggressiveness.

 

## Determining aggressiveness in the field

The aggressiveness of several isolates can be compared within a growing crop by assessing the rate at which primary foci spread via multiple cycles of infection and sporulation. This has been achieved using separate field plots for each isolate but is restricted to the early stages of the epidemic as the isolates rapidly spread from plot to plot (Spielman *et al*., 1992). An alternative approach, simulating infection under commercial conditions, is to infect field plots with a mixture of several *P. infestans* isolates and use a 'mark and recapture' approach to monitor their spread in the subsequent epidemic. Blight lesions are sampled at several time points and the isolates discriminated (for example by genetic fingerprinting) to estimate the frequency and thus 'success' of each. Such studies have been undertaken but the relatively time consuming and expensive isolate discrimination has proved a limiting factor (Legard *et al*., 1995; Lebreton *et al*., 1999). However, the recently developed simple sequence repeat (SSR) fingerprinting markers (Lees *et al*., 2006) are ideal for such a procedure (Cooke & Lees, 2004) and pilot studies (Shaw *et al.*, 2006) have recently shown that SSR markers allow the rapid and relatively cheap discrimination needed. Such methods can be applied to pathogen DNA extracted from a lesion without the need for isolation of the organism onto agar.

Using this method all the components which can be assessed in the laboratory can be measured under a range of field conditions and a range of factors such as cultivar and location could be examined as a means of studying the interactions between aggressiveness and other factors.

## Measurement of aggressiveness on tubers

Freshly harvested, washed tubers are inoculated with known numbers of spores, kept wet for 24 h and incubated for at least 10 d when incidence of blighted tubers is scored. Scoring may be repeated a second time to detect slow development of symptoms. Frequencies can be used to calculate the average number of infections per tuber from the Poisson Distribution or from Gregory's Multiple Infection Transformation (Flier *et al.*, 1998). Some studies include an assessment on a 1 – 5 scale of the severity of tuber blight from longitudinal sections of tubers. A severity index has been calculated from the product of the logarithm of infections per tuber and the severity score (Flier *et al*., 1998).

The ability of a particular isolate to infect tubers *in situ* in a field crop before harvest can be measured immediately after healthy plants have been defoliated. Large volumes of counted spore suspension are used as a drench over the top of the ridge and frequencies and severities can be assessed as above. The method is rarely used as it is time consuming to prepare enough inoculum (see EUCABLIGHT protocol 'Field test for tuber blight resistance' at www.eucablight.org/Upload/Eucablight/Protocol/TuberField_V12.pdf).

There are many pitfalls in carrying out comparisons of aggressiveness. There is clearly room for improvement and upgrading of the methods used in future studies. Where possible, methods allowing infection and growth of isolates which mimic what happens in the field should be adopted. A challenge in comparing results is that methods used by different workers are rarely standardised.

 

# 4. Foliar aggressiveness

## *Within and between populations of* P. infestans

It is on the aerial parts of the potato crop that *P. infestans* inoculum predominantly builds up and spreads, and for this reason aggressiveness to the foliage is the aspect most commonly studied. Blight is present in almost all areas where potatoes are grown and *P. infestans* population displacements at both local and international scales have been reported over time. In many cases, the changes are attributed to increased aggressiveness but few studies have investigated this.

### The early years

Most studies have compared relative levels of some components of aggressiveness amongst isolates or populations of *P. infestans*. In early reports, isolates were discriminated by race (i.e. virulence) as that was the only criterion available. Thurston (1961) for example, conducted studies in which different races were co-inoculated onto susceptible hosts and a sample of isolates from the resultant lesions were race tested. This illustrated an increased survival of a race 0 isolate over those of other more complex races. A limitation of such studies is that one could not be sure that virulence (i.e. the trait defining race) would be stable over the period of the experiment and objective comparisons between studies were not possible since isolates of the same race may have had a very different genetic background. Further details on the associations between virulence and aggressiveness are covered later in the review. Tooley and Fry (1985) conducted a key early study comparing aggressiveness of six isolates in field trials of a single susceptible potato variety at two locations. Differences in aggressiveness were only observed in a trial in which the environmental conditions were sub-optimal for disease development. They thus highlight the complexity of the interactions that need to be considered when estimating aggressiveness. In both of the above studies it can be assumed that a single clonal lineage of the pathogen was involved and this highlights the differences apparent even within a population. This was further demonstrated in a very detailed series of laboratory experiments in which considerable variation in aggressiveness amongst single zoospore isolates of typical field isolates was demonstrated (Caten & Jinks, 1968; Caten, 1970).

### The 'new' populations

The appearance of new A1 and A2 strains in Europe and the US prompted a series of surveys that demonstrated the displacement of the previous *P. infestans* population, at first from Europe over the mid to late 1980s and 1990s (Spielman *et al*., 1991; Drenth *et al.*, 1993; Goodwin & Drenth, 1997) and subsequently from the US (Fry *et al.*, 1993; Goodwin *et al*., 1998). Over the early phase of population change, it was generally accepted that increased aggressiveness explained the displacement of the old population (e.g. Spielman *et al.,* 1991) with few studies to support the hypothesis. One of the first studies (conducted by necessity in growth rooms) compared the aggressiveness of isolates of the US-1 clonal and Mexican sexual populations (Tooley *et al.*, 1986). The Mexican isolates had more complex races but were not significantly more aggressive. Their infection efficiency was lower but they caused larger lesions than the US-1 isolates. Since the US industry had been forewarned by events in Europe, the spread of the new lineages (termed US-7, US-8 etc) was tracked in greater detail

 

and the increased disease severity was apparent to growers (e.g. reviewed by Fry & Goodwin, 1997).

Studies examining the factors driving the population followed and, in general, supported the assumption that increased aggressiveness explained the displacement. These papers are summarised below.

## Population changes in the USA

Kato *et al*. (1997) examined the aggressiveness and fungicide sensitivity of many isolates of the US-1, US-7 and US-8 lineages and concluded that the former explained differences in their incidence in US potato crops. On average, isolates of the previously dominant lineage, US-1, had the longest latent period, smallest lesions and least sporulation compared to those of new lineages US-7 and US-8. Marked variation in all these factors was also noted within each lineage. The lesion expansion rate of US-1 and US-8 data was projected onto a simulation model which indicated that, on average, fungicide applications every 7 days would be needed to control US-8 compared to every 9-10 days for US-1.

Mizubuti and Fry (1998) examined the sporangial germination and aggressiveness parameters of new (US-7 and US-8) and old US (US-1) lineages at different temperatures. They indicated that there was no interaction between temperature and the fitness components of the lineages. General differences such as the US-7 lineage having a shorter incubation period (IP) and US-8 having a more rapid lesion expansion rate than US-1 isolates were commented on but, overall, no single lineage had an apparent pathogenic advantage in all attributes. Differences in sporangial germination may however be significant. A significantly lower overall percentage germination of sporangia of the new lineages was noted at 15-20°C compared to US-1 suggesting the new lineages were less well adapted to higher temperatures. However germination measured at 2, 4, 6 and 8 hours into the assay at 15°C indicated that significantly more US-7 and US-8 sporangia had germinated by 2 hours. This increased germination rate at the early time points may confer an advantage in periods of changeable weather with shorter 'windows' of optimal infection conditions.

Miller *et al*. (1998) compared isolates of several US lineages from the Columbia basin on four cultivars and observed considerable variation and strong cultivar-isolate interactions. Isolates of US-8 and US-11 were typically more aggressive than other genotypes as measured by AULEC. The results supported the shift in population from US-1 to US-8 isolates in the Columbia Basin being related to increased aggressiveness rather than the fact that US-8 is resistant to metalaxyl.

The previous studies were based on laboratory assays, but the same conclusions were drawn from a field study in which field plots of Russet Burbank were infected with a mixture of both US-1 and US-8 isolate inoculum (Miller & Johnson, 2000). Lesions were sampled from the subsequent epidemic and genotyped by isozyme analysis. Over 550 isolates were examined in epidemics over two seasons and only 1% of the isolates were of the US-1 genotype. Field studies such as these involve several cycles of infection and thus examine components of fitness and aggressiveness.

 

Recently, Young *et al.* (2005) investigated competition between six US clonal lineages including US-1 and US-8 in field trials in Michigan using four potato cultivars with differing levels of foliage blight resistance exposed to infection from inoculated spreader rows. In these trials, US-8 proved by far the most aggressive with very few other genotypes being detected regardless of cultivar.

## Population changes in Europe

In Europe, a detached leaf bioassay was used to compare the aggressiveness of 36 isolates sampled from three regions of the Netherlands in 1995. Significant variation was observed within regions but no consistent differences between the three regions sampled. A slightly longer mean latent period was noted amongst isolates from a region of allotment gardens that may have been related to the presence of tomato-adapted isolates (Flier & Turkensteen, 1999). All the isolates in this study were representative of the 'new' lineage and this may explain the absence of any clear geographic clustering of isolates on the basis of aggressiveness.

Zwankhuizen & Zadoks (2002) in examining the longer-term impact of blight in the Netherlands suggest that increased blight intensity and unpredictability have coincided with the appearance of the new population. A study of aggressiveness of 'new' population isolates from Nordic states (Hannukkala, personal communication) similarly reported variation but no clear clustering on the basis of country of origin.

In France, Lebreton *et al*. (1999) compared the aggressiveness of 44 isolates of *P. infestans* from potato and tomato on detached leaflets of both hosts and showed a wide range of aggressiveness particularly among the potato-derived isolates when tested against potato. These authors also showed good agreement between results from detached leaflet and whole plant experiments. In a more recent study in France, Montarry *et al*. (2006) examined components of aggressiveness of 139 isolates recovered from three cultivars grown at a single site over two years. Variation in aggressiveness was observed and the most aggressive isolates were from cv Bintje rather than Désirée. Two hypotheses were presented to explain this: a larger population size on the susceptible Bintje may have increased the chances of selection of aggressive forms or it may have been related to prior adaptation to Bintje which is widely grown in the region compared to Désirée.

In Poland, a long-term study of *P. infestans* isolates collected between 1987 and 2001 observed a marked increase in the percentage of isolates categorised as 'very aggressive' between 1987 and 1992 (Zarzycka *et al*., 2002). In subsequent years this figure remained similarly high (between 66 and 100%).

Two studies have compared isolates from different lineages. Carlisle *et al*. (2002) examined many components of aggressiveness of strains of US-1 and US-8 lineages as well as a Mexican isolate in their comparison of 20 isolates from Northern Ireland against three cultivars. They demonstrated highly significant differences amongst the Northern Irish population with more marked differences apparent on the more resistant cultivars Cara and Stirling. No single isolate performed best for all parameters measured but a group of 7 isolates were consistently the most aggressive. They also demonstrated that the US-1 and Mexican isolates were consistently less aggressive against all tested cultivars. However, neither of these isolates was originally from potato which may have influenced the results.

 

The US-8 isolate was particularly aggressive on cultivar Cara but not against Bintje and Stirling. This study highlights the need to conduct such studies against a number of cultivars and especially cultivars with moderate to high levels of race non-specific resistance.

The most comprehensive isolate collection studied was of 618 UK isolates against three potato cultivars (Maris Piper, Cara and Stirling). In this study, Day & Shattock (1997) provided some evidence to suggest that the 'old' population was not as aggressive as the 'new'. Again a range of aggressiveness was demonstrated but the differences between isolates of the 'old' and 'new' were not striking. This may have been because IF and mean numbers of sporangia per lesion were combined into 'fitness index' and sporulation, particularly at a fixed time point, has not proved the most useful character for discriminating isolates in other studies. Despite the authors care to limit comparisons to isolates recovered in the same cropping season, the inevitable problem of older isolates having been in culture for longer and the limited number of 'old' isolates in culture collections makes it difficult to draw firm conclusions on GB populations on the basis of this single study.

In a field study of competition between *P. infestans* isolates representing six genotypes from the current Northern Ireland *P. infestans* population, Young *et al.* (2005) showed very marked selection for particular genotypes among the four different potato cultivars used and this was most marked with the more field-resistant cultivars (Milagro and Stirling). Young *et al.* (2006), using detached potato leaflets, showed that this host-pathogen interaction was influenced by inoculum concentration and could not be explained by the general aggressiveness of individual genotypes. This suggests that aggressiveness testing alone will not reveal how the *P. infestans* population is influenced by the potato cultivars grown and that studies of competitiveness are also required.

Studies at SCRI were conducted to investigate the relative aggressiveness of four isolates used to infect field plots of five cultivars representing a range of blight resistance (Bintje, Désirée, Pimpernel, Teena and Stirling). Each single-cultivar plot was inoculated with equal amounts of each of four new-population isolates and foliar lesions sampled at four time points throughout the epidemic. DNA-based simple sequence repeat (SSR) fingerprinting was used to track the frequency of each isolate. Clear differences in isolate frequency were demonstrated and strong cultivar-isolate interactions observed, especially on the more resistant cultivars (Alison Lees and David Cooke, unpublished).

A limited number of GB isolates, collected via the BPC Fight Against Blight campaign in 2004, were tested for response to temperature, virulence, lesion growth rate and sporulation on leaves and tuber infection. The results indicated considerable variation in most traits likely to affect their aggressiveness to GB crops. A further study in 2005 similar to that described above from SCRI, tracked the frequency of three isolates in a field epidemic of cv. Nicola. Although the sample number was relatively small (67 isolates), SSR fingerprinting indicated clear differences in isolate frequencies suggesting differences in aggressiveness or fitness amongst the three isolates (Shaw *et al.*, 2006). A within-crop weather station was used and blight progression in the absence of Smith periods was clearly demonstrated.

 

*P. infestans* strains within populations vary in their aggressiveness to foliage. There is clear evidence of increases in aggressiveness in US *P. infestans* populations. In Great Britain there is circumstantial evidence to suggest that the new population now present is more aggressive than the one it displaced which was prevalent up to the late 1970s, but conclusive evidence is lacking. However, this is academic as growers have been living with the new population for over 25 years. It is more important to know whether the situation in GB is changing now and whether *P. infestans* strains in other regions of Europe, particularly those from which we import seed and ware potatoes differ in aggressiveness from those currently present here. This is particularly important in terms of the marked increase in A2 mating type observed in the 2005 FAB campaign and population changes being monitored in the current BPC Blight survey project.

## *Variation in aggressiveness in relation to sexual recombination?*

In the USA, Mayton *et al.* (2000) generated progeny of US-17 and US-8 lineages but found that, unlike the US-8 parent, none of 10 progeny tested in the field were capable of initiating an epidemic. Gavino *et al*. (2000) however, provided strong evidence for genetic recombination between two lineages of *P. infestans* in the Columbia Basin (US-6 and US-7) generating an aggressive new lineage (US-11) responsible for a severe disease outbreak in tomato and potato crops in California in 1998. This demonstrates that one of the fears of sexual recombination, the generation of new aggressive genotypes, has been realised in the United States. In areas of Europe such as the Nordic countries where sexual recombination is occurring (Brurberg *et al*., 1999) evidence for increases in aggressiveness is limited as no studies have compared the previous and current populations. Similarly within the Netherlands where high levels of the A2 mating type have also been reported (Zwankhuizen *et al*., 2000) no comparisons have been made.

Sexual recombination can sometimes produce new, more aggressive strains of *P. infestans*, but this has not been clearly demonstrated in Europe. We do not know if *P. infestans* strains in sexually-reproducing populations such as that in the Netherlands are more aggressive than those currently present in Great Britain or whether the recent upsurge in the incidence of the A2 mating type in Great Britain is associated with increased aggressiveness.

## The relationship between mating type, fungicide resistance, virulence and aggressiveness

*P. infestans* often functions as a highly clonal organism, reproducing mainly asexually, thus chance associations between aggressiveness and other characters such as mating type and fungicide resistance often arise and are perpetuated for as long as the clonal lineage is prevalent. These traits are however not truly linked but are simply chance associations that are often misinterpreted and result in questionable claims such as ‘A2 is more aggressive’. In this section we critically examine evidence for such associations.

 

i) Mating type

The modification of the effect of one gene by one or more other genes, is termed epistasis. While it is theoretically possible that the gene(s) associated with mating type indirectly affect aggressiveness, such epistasis is far less likely than the chance associations between mating type and aggressiveness discussed above. O'Sullivan *et al.* (1995) noted an association between the A2 mating type and cv. Cara, with 46% of A2 isolates being recovered from this cultivar in the Republic of Ireland in the years 1988-94; these isolates were derived from tubers (Dowley, L.J., personal communication). Subsequent experiments showed that these isolates were unusually aggressive to tubers of cv. Cara whereas they exhibited normal aggressiveness to Kerr's Pink, suggesting that the aggressiveness was cultivar specific (Dowley *et al.,* 1991). Day & Shattock (1997) found little difference between the A1 and A2 isolates examined in their study although they did comment that A2 isolates were more aggressive on detached leaflets of cv. Cara than the A1 isolates tested. Carlisle *et al*. (2002) also discussed the behaviour of the A2 mating type on cv Cara. The A2 US-8 isolate was more aggressive than other isolates on cv. Cara than on cvs. Bintje or Stirling, but this most probably related to the genetic background of the lineage rather than specifically to its mating type. Flier & Turkensteen (1999) showed no association of aggressiveness and mating type amongst a selection of 37 Dutch isolates collected in 1995. Suassuna *et al*. (2004) examined the aggressiveness and host specificity of the A1 US-1 and the A2 BR-1 lineages in Brazil but did not consider mating type as a factor.

ii) Fungicide resistance

Kadish and Cohen (1988 & 1989) conducted a series of competition experiments in which inoculum of metalaxyl-sensitive and resistant isolate pairs at a 9:1 ratio were used to initiate epidemics in polytunnel plots in the absence of the fungicide. Throughout the resultant epidemics lesions were collected at random and scored for metalaxyl sensitivity in a leaf disk assay. They concluded that the metalaxyl-resistant isolates were fitter, as the 10% starting proportion increased in all cases to 70-80% by day 16. In three of the six comparisons the frequency of the metalaxyl-resistant isolates decreased again later in the epidemic and this was proposed to be as a result of increased infectious period and sporulation capacity of the metalaxyl-sensitive isolates in each case. The genetic background of the resistant and sensitive isolates is not reported. A difference in aggressiveness is apparent, but one cannot conclude that the metalaxyl resistance is responsible for the differences without further evidence. In a study in the UK (Day & Shattock 1997), metalaxyl-resistant isolates were, on average, less aggressive than sensitive strains. Such responses have been reported elsewhere (Dowley & O'Sullivan, 1985). Dowley (1987) showed that metalaxyl-resistant isolates collected in 1984 produced fewer sporangia per unit area than the sensitive ones, but when such a comparison was carried out again some years later, this association between metalaxyl resistance and reduced sporulation had disappeared (Dowley, L. J., personal communication), suggesting that selection had occurred within the resistant population. In the US, Miller *et al*. (1995) reported that in a group of 30 isolates those that were metalaxyl-resistant were not significantly more aggressive than the sensitive isolates. In general, it appears that metalaxyl-resistant isolates of *P. infestans* express equal or greater foliar aggressiveness than sensitive ones (Gisi & Cohen, 1996).

 

Kato *et al.* (1997) examined sensitivity to chlorothalonil and mancozeb of a range of isolates from US lineages and although some differences were observed this was not sufficient to explain the dominance of particular lineages.

iii) Virulence

No association between aggressiveness and virulence was found by Flier & Turkensteen (1999) or by Tooley *et al.* (1986). Andrivon (1994) and Peters *et al.* (1998) studying populations in France and Canada respectively, went further, stating that specific virulences appeared to be behaving as neutral characters and that, in Canada, the spread of US-8 was consistent with a highly aggressive strain which just happened to also have a complex combination of virulences. Another study (Pilet *et al.*, 2005) specifically examining aggressiveness of isolates virulent against R2 in France indicated that the mutation to virulence against R2 did not reduce pathogenic fitness.

iv) Other factors

Interactions between temperature optima and traits related to aggressiveness could be significant (Mizibuti & Fry, 1998). Although not directly related to aggressiveness this would be important data to have on UK populations. Parameters defining Smith periods may need to be changed as discussed in Nordic states where adjustments in the Førsund rules were examined to evaluate reducing the minimum temperature from 10 to 8°C (Hermansen & Amundsen, 2003).

There is no evidence for consistent associations between aggressiveness and mating type, fungicide resistance or virulence. However, other traits such as temperature range and optima will have an indirect effect on blight aggressiveness under specific conditions and therefore it would be valuable to have such information for new genotypes found in the UK FAB campaign and associated blight survey project.

 

# 5. Tuber aggressiveness

## *Within and between populations of* P. infestans*:*

Studies of aggressiveness of *P. infestans* to tubers are considerably fewer than those to foliage.

Caten (1974) reported small, but significant, differences amongst six isolates of the 'old' clonal lineage in their ability to infect tubers. Flier *et al.* (1998) tested isolates collected from three regions of the Netherlands in 1995 and compared them with three reference isolates from the old population (two of which were isolated in the 1950s) used in determining tuber resistance ratings for the Dutch official lists. The study demonstrated significant variation amongst the 1995 isolates but no differences in the average tuber aggressiveness between regions. However, the reference isolates were significantly less aggressive and a negative correlation between number of virulence factors and tuber aggressiveness was noted in one region. This is the only record of such a link between virulence and aggressiveness and there is no clear explanation for it. Overall, no correlation between tuber and foliage aggressiveness or association between tuber aggressiveness and mating type or virulence factors was observed. Components of tuber resistance were examined and no correlation between infection and subsequent spread within the tuber was observed. The study provided clear evidence of increased aggressiveness to tubers in the new populations compared to the 'old' lineages and highlights the need to ensure official resistance scores are derived from tests with appropriate and aggressive isolates.

In the US, Lambert and Currier (1997) showed that isolates of lineages US-6, US-7 and US-8 caused tubers to rot faster than US-1 isolates. Similarly, in Canada, Peters *et al*. (1999) showed that most isolates of recently introduced genotypes were more aggressive to tubers than the US-1 isolates but that there was variation between isolates of the same lineage. Cultivars with higher levels of resistance were more badly blighted by the new lineages. They also observed that wounding the tubers obscured the differences between the lineages implying that the differences may be related to tuber infection rather than tuber tissue colonisation.

Marshall and Stevenson (1996) noted a clear increase in sprout infection after tuber inoculation with US-8 compared to US-1 isolates. A large increase in seed piece decay was also observed with US-8 isolates. It is difficult to generalise about the impact of the aggressiveness of isolates on their over winter survival in tubers, especially seed tubers, and spread the following season. Many seed crops are now stored under refrigerated conditions (*c*. 3°C) and at such low temperatures, differences in the rate of tuber rotting between isolates will be small. It is likely that isolates more aggressive to foliage will infect tubers earlier in the growing season. Tubers infected earlier are less likely to survive until the following spring because of decay, or the appearance of obvious symptoms, by the time of harvest or between harvest and grading. Although there are limited data on the subject, isolates that rot planted seed tubers faster are more likely to prevent plant emergence. This could reduce, or at least delay, the contribution of more aggressive strains to the foliar epidemic initiated by infected seed.

 

The phenomenon of lesion arrest, in which blight development on tubers is limited to thread-like lesions, has been reported by several authors (Lacey, 1967; Lapwood, 1967; Pathak & Clarke, 1987). Lesion arrest occurs in cultivars with high levels of race non-specific resistance and also in susceptible ones (Wastie, 1991). There is no information on whether the frequency of lesion arrest is related to aggressiveness. The role of arrested lesions in further disease development is unclear, presumably because it has not been studied in detail. One report states that the pathogen is latent in these lesions but it is rarely re-activated (HE Stewart, unpublished, in Wastie, 1991).

## *Variation in aggressiveness in relation to sexual recombination?*

There are no reports that specifically examine the variation in tuber aggressiveness associated with sexual recombination.

## *The relationship between mating type, host resistance, fungicide resistance and tuber aggressiveness*

Dowley *et al*. (1991) showed that A2 isolates derived from Cara tubers were unusually aggressive to tubers of this variety whereas they exhibited normal aggressiveness to the variety Kerr's Pink. Flier *et al*. (1998) found no relationship between mating type and aggressiveness on tubers.

Flier *et al*. (1998) provided clear evidence of increased aggressiveness to tubers in new populations compared to the 'old' lineages and highlighted the need to ensure official resistance scores are derived from tests with relevant aggressive isolates. Subsequently, Flier *et al*. (2001) examined the interaction between nine potato cultivars and five isolates of *P. infestans* (two from the old and three from the new population in the Netherlands). They found marked differences in the responses of tubers to the different isolates and concluded that the assumed stability of tuber blight resistance should be re-considered in the light of the differential interactions observed. In other words, the possibility of selection for tuber aggressiveness against a particular cultivar, as a result of widespread use of that cultivar, should be considered. This is supported by the earlier findings of Bjor & Mulelid (1991) who reported that tubers of the field-resistant cv. Pimpernel were more susceptible to infection by isolates from Pimpernel crops than those from other cultivars suggesting that adaptation had broken down tuber resistance.

Dowley (1987) and Grinberger *et al.* (1995) found no difference in the percentage tuber infection between phenylamide-resistant and –sensitive isolates, although Grinberger *et al.* (1995) reported that the resistant isolates produced larger lesions and concluded that they caused more severe infection than the sensitive ones. Walker and Cooke (1988, 1990) showed that phenylamide-resistant isolates from Northern Ireland grew faster on tuber slices at 5°C than –sensitive ones and found that fewer tubers inoculated with resistant than with sensitive isolates survived to produce plants the following season. They suggested that this resulted in fewer resistant than sensitive isolates surviving the winter; this may help to explain the lower occurrence of resistant strains at the beginning of the season than at the end of the preceding one as noted by Gisi and Cohen (1996) and Dowley *et al.* (2002). Similarly, Kadish and Cohen (1992) recovered more phenylamide-sensitive than –resistant isolates from tubers inoculated with individual isolates after 20 weeks and also more –sensitive than –

 

resistant isolates from dual-inoculated tubers. They concluded that this explained the higher incidence of sensitive strains in the first foci in commercial fields at the beginning of the season and attributed the loss of phenylamide-resistant strains to their greater aggressiveness to tubers.

Bashan *et al*. (1989) observed that phenylamide-resistant isolates had a significantly faster initial rate of zoospore release than phenylamide-sensitive isolates. This could influence the ability to infect tubers when environmental conditions suitable for tuber infection are short-lived.

*P. infestans* strains within populations vary in their aggressiveness to tubers. There is evidence from The Netherlands suggesting that the new population now present in Great Britain is more aggressive on tubers than the one it displaced. The assumed stability of tuber blight resistance in cultivars should be re-considered in the light of the differential interactions observed between host and pathogen. As is the case for the foliar studies it is important to know whether *P. infestans* strains in other regions of Europe, particularly those from which we import seed and ware potatoes, differ in aggressiveness from those currently present in Great Britain and whether the situation in Great Britain is changing. This is particularly important in terms of the marked increase in A2 mating type observed in the 2005 FAB campaign and population changes being monitored in the current BPC Blight survey project.

 

# 6. Links between host specificity and aggressiveness

## *What is the risk of adaptation of* P. infestans *to cultivars with non host-specific resistance to late blight?*

The possibility that selection yields *P. infestans* strains with specific aggressiveness to particular cultivars with race non-specific resistance has been considered by various authors and some have suggested that the risk of this is greater since the introduction of new *P. infestans* populations. Interpretation of some of the findings is complicated by the lack of information on the presence of R-genes in many cultivars so that it is difficult to exclude the possibility that some of the apparent increased aggressiveness may be due to selection for virulence to both known and unknown specific R-genes.

Tests that indicate an increase in disease caused by a specific isolate on a cultivar as a result of prior 'training' on that cultivar indicate a degree of host adaptation. The logical consequence of this process would be erosion of host resistance. Several examples of such adaptation have been reported in the literature (Hussain, 2002; Caten, 1974), whereas other studies have found no evidence for it (James & Fry, 1983; Montarry *et al*., 2006). Such studies were largely run with isolates from 'old' populations and it is possible that more significant differences may be evident in more rapidly evolving current European lineages. Jinks and Grindle (1963) and Hussain (2002) report clear examples of specific adaptation of isolates from a particular cultivar to that same cultivar after successive transfers, or 'training', on tubers or leaves of that cultivar in the laboratory. Specifically, Hussain (2002) exposed two isolates from the Scottish *P. infestans* population (Cooke *et al*., 2003) to eight successive rounds of infection of detached leaves of each of 4 cultivars. At the end of this cultivar-specific training period each isolate was tested on its 'own' and the 'other' three cultivars. Lesion size data indicated a statistically significant increased lesion size on each isolates 'own' cultivar compared with that on the 'other' cultivars. This indicates that even in the absence of any mutation or sexual process cultivar adaptation may occur. This adaptation was lost after culture on agar for only six weeks.

In Norway, Bjor & Mulelid (1991) investigated unusually severe late blight on tubers of cv. Pimpernel, which is believed to lack R genes, and showed that isolates from this cultivar caused more tuber infection on Pimpernel than on other cultivars. In contrast, Vanderplank (1971) reported a stable ranking of cultivar resistance in the Netherlands over a 30 year period. Similarly, Colon *et al.* (1995) investigated the resistance of 22 R gene-free cultivars introduced between 1900 and 1954 in field trials over three years (1991-1993) and found no evidence for erosion of resistance i.e. specific aggressiveness of *P. infestans*. Flier & Turkensteen (1999) comment that it is possible that adaptation occurs, but it is likely to be specific adaptation of a *P. infestans* genotype to a particular variety rather than a general increase in aggressiveness. Flier *et al*. (2003) examined the stability of partial (race non-specific) resistance in tubers and foliage over two years and identified clear cultivar x isolate interactions. These authors suggested that adaptation of isolates to race non-specific resistance is far more likely to occur in areas with sexually reproducing *P. infestans* populations compared with clonally reproducing ones. This may be of concern in the light of the increased incidence of the A2 mating type in *P. infestans* populations in Great Britain. Inglis *et al.* (1996) compared resistance rankings of cultivars in response to foliar infection by

 

isolates of *P. infestans* from the new US population with those previously obtained with the old population and found them to be nearly identical, suggesting that erosion of partial resistance was not important in the US. Grünwald *et al.* (2002) reported that potato cultivars from the Mexican national breeding programme have maintained their resistance in the field for many years, but in Minnesota, Jenkins & Jones (2003) found that some US cultivars which were considered to be resistant to the US-1 clonal lineage were susceptible to US-8. An international evaluation of the stability of resistance to late blight in potato conducted over 11 countries (Forbes *et al.*, 2005) found that overall resistance to *P. infestans* was robust and resistant potato genotypes were basically stable in all locations and trials, suggesting a lack of specific adaptation of *P. infestans* to particular potato cultivars.

The risk of selection of *P. infestans* strains with aggressiveness to potato cultivars with race non-specific resistance appears low as, in general, such cultivar resistance appears stable. There are however exceptions (e.g. Pimpernel, Cara) and there is experimental evidence that strains can gain specific aggressiveness through 'training'. For the purposes of appraising risk, and therefore disease management, cultivar resistance changes need to be quantified where significant cultivar x isolate interactions are shown to occur for race non-specific resistance. For example, a decline in cultivar resistance rating from 7 to 6 (on the 9-point scale) has fewer implications for disease management than a decline from 7 to 3.

## *Do local or imported pathogen populations from other solanaceous hosts such as tomato or weeds/crops/ornamentals pose new threats?*

i) Aggressiveness to tomato

There is a substantial body of literature showing that *P. infestans* strains may differ in their aggressiveness to potato and to tomato. In general, isolates obtained from tomato are more aggressive on tomato than potato and *vice versa*, although the difference is qualitative, both potato and tomato being capable of being infected to some extent by isolates from either host (e.g. Vega-Sanchez *et al.*, 2000; Suassuna *et al.*, 2004; Garry *et al.*, 2005). Lee *et al.* (2002) reported a genetic locus with a strong influence on tomato aggressiveness, with low aggressiveness to tomato being dominant. In the field, *P. infestans* strains on potato and on tomato often behave as separate populations comprised of distinct genotypes with apparently little exchange of genetic information even when they are in close proximity. In some regions of Europe there is evidence for a greater incidence of the A2 mating type strains on field-grown tomato than on potato (Lebreton & Andrivon, 1998; Knapova & Gisi, 2002). Could *P. infestans* strains from tomato pose a risk to potato crops in the UK? Commercial tomatoes are not grown outdoors in UK but those grown in gardens or allotments are sometimes infected by late blight. There is thus a tomato *P. infestans* population which could potentially function as a reservoir for genetic variation and endanger potato but this has not been examined in detail.

 

ii) Aggressiveness to other solanaceous hosts

Solanaceous weed and ornamental species may sometimes be infected with late blight and, where they grow in proximity to potato crops, might potentially act as reservoirs of inoculum or aid generation of diversity if they permit contact between crop-specialised genotypes. Weeds and ornamental plants belonging to the family Solanaceae occur very widely. In the UK, *Solanum nigrum* (black nightshade) often grows in the close vicinity of potato crops and *S. dulcamara* (woody nightshade, bittersweet) in hedgerows, yet reports of natural infection of late blight of these plants are infrequent. Although *P. infestans* is capable of infecting a wide range of solanaceous hosts (Erwin & Ribeiro, 1996), particularly when artificially inoculated (Dandurand *et al*., 2006), natural infection of non-crop Solanaceae has seldom been observed in the field. However, with the introduction of the new *P. infestans* population, there is a concern that the situation might change and that strains more aggressive to weed species could pose a threat to potato crops.

During a nationwide late blight survey in the Netherlands in 1999 and 2000, infection of black nightshade by *P. infestans* was observed on several occasions, but was considered to be a relatively rare event and not to contribute to the overall disease pressure (Flier *et al*., 2003). In Henfaes Research Centre, North Wales, black nightshade plants infected by *P. infestans* were found within a naturally-infected potato blight trial in 1999 (Deahl *et al.,* 2004), but although late blight has occurred in potato plots at Henfaes every year since 1999 and the black nightshade weeds have been regularly monitored, only in 2004 were a few *P. infestans*-like lesions again observed. Late blight was only found when the disease was already widespread in potato and the *P. infestans* genotypes isolated were typical of those infecting local potatoes (Deahl *et al.*, 2006) so it appeared that infection of *S. nigrum* did not increase the risk to potato.

*Solanum dulcamara* (woody nightshade) is the only perennial nightshade occurring wild in the UK and therefore might potentially act as an overwintering host for *P. infestans.* There is only one British record, from Harpenden, England (Hirst & Steadman, 1960), but infection was observed more recently in Northern Ireland, when leaf lesions of *P. infestans* were found on a woody nightshade plant growing as a weed in Belfast in 1998 and subsequently in 1999 (Cooke *et al.,* 2002) and 2002. The 1998 isolates were all of the Northern Ireland *P. infestans* genotype which is commonest on potato, but isolates characterised from 1999 and 2002 shared a different genotype, one which is relatively rare on potato, suggesting that a degree of host adaptation to woody nightshade might be involved (Cooke *et al.*, 2006). Infection has not been seen since 2002 and has only been found when blight is already widespread in potato fields, so there is no evidence to suggest that woody nightshade could act as an overwintering host in Great Britain or Ireland. Flier *et al.* (2003) found infection of *S. dulcamara* in his 1999-2000 survey in the Netherlands, but considered it to be relatively rare, while mentioning the possibility that the establishment of genetic variation in the pathogen population through sexual reproduction may be broadening its host range.

Flier *et al.* (2003) also reported infection of *S. sisymbriifolium*, which was being evaluated as a potential trap crop for potato cyst nematodes, and had previously been regarded as a non-host for *P. infestans*. Test plots in the UK should be carefully examined and any infection reported via the BPC FAB campaign. Flier *et al.* (2003) commented that testing randomly collected *P. infestans* isolates from the major hosts for their ability to infect potential new hosts was not the most efficient method for establishing host/non-host status and proposed

 

exposing a number of genotypes of a potential host to a variable pathogen population, preferably under field conditions.

Solanaceous ornamental plants may also be infected by *P. infestans*, but reports are infrequent. Petunia was first reported to be infected by blight in the UK in 1956 (Hirst and Moore, 1957), but despite the widespread use of petunia as bedding and container plants, there seem to have been no further UK reports. However, there have been recent outbreaks of blight on petunia in the US (Deahl & Fravel, 2003; Becktell *et al.*, 2005a, Becktell *et al*., 2005b), suggesting that petunias are very susceptible to current US *P. infestans* genotypes and could have a role in the epidemiology of potato blight. During an extensive tomato late blight outbreak in 1998 (Gavino *et al*., 2000), a commercial production greenhouse in California lost both petunias and tomatoes to late blight. Subsequently, there were occurrences of late blight on field-grown tomatoes in New York for which late blighted petunias were thought to have been the main source of inoculum (Smart & Fry, 2001). Most recently *P. infestans* has been reported to cause leaf blight on petunia in the north-eastern United States (Deahl & Fravel, 2003); isolates from an outbreak in 2002 and 2003 were shown to be US-8 and identical to isolates from nearby potato crops. Such petunia-aggressive strains of *P. infestans* could cause problems in the UK for both bedding plant and potato production.

Thus, although *Solanum* spp. weeds including *S. nigrum* and also *S. dulcamara* can act as hosts of *P. infestans*, they are generally only infected when late blight is widespread and by pathogen genotypes already occurring in potato crops in the same area. The basis of the resistance of these species to *P. infestans* is not well understood although Flier *et al.* (2003) suggested that *S. nigrum* may contain R-genes. It appears unlikely that these *Solanum* weeds contribute significant inoculum or diversity to late blight epidemics. Nonetheless, even infrequent infection of *Solanum* weeds by *P. infestans* warrants investigation, particularly since some of these species have been used in potato breeding as sources of supposed non-host resistance (e.g. Zimnoch-Guzowska *et al*., 2003).

Other Solanaceous plants including *Solanum* weeds and cultivated species such as petunia are sometimes infected by late blight. The evidence to date seems to suggest that blight is spreading from potato to other species and thus such species are not involved in any increases in aggressiveness. This should however be monitored in light of a changing pathogen population and if *S. sisymbriifolium* becomes a common biocontrol for PCN in GB. Blight is common on tomato and potato grown in gardens and allotments in the UK and although such sites may pose a risk as a source of inoculum and of genetic recombinants no clear influence on aggressiveness has been demonstrated to date.

 

# 7. Overview of the possible mechanisms governing aggressiveness

Despite many records of clear differences in pathogen aggressiveness to foliage and tubers there have been relatively few studies focussed specifically on understanding the mechanisms. Of course there have been tremendous gains in *P. infestans* genomics and gene discovery (Randall *et al*., 2005) particularly with the recently released draft *P. infestans* genome sequence (Chad Nusbaum, personal communication (www.broad.mit.edu)). Such studies are accelerating the progress towards understanding the mechanisms and processes of *P. infestans* pathogenicity. A key target of such fundamental research is to understand infection and plant defence with a goal of stable and durable host resistance. Much effort is being devoted to the localisation (van der Lee *et al*., 2001; Whisson *et al*., 2001) discovery (Armstrong *et al*., 2005) and functional analysis (Kamoun, 2006) of avirulence genes that govern the highly specific interactions with single potato R-genes. The most promising leads towards genes involved in more general aggressiveness have come through the description of a class of *Phytophthora* extracellular proteins termed PEX genes (Torto *et al*., 2003). Among these are candidates for factors controlling aggressiveness (see reviews Birch *et al.*, 2006, Kamoun, 2006). One example, a phytotoxin-like *scr74* gene (Liu *et al.*, 2005), was found to be a member of a remarkably diverse and rapidly evolving gene family under diversifying selection consistent with a role in the infection process. Another recent study has identified an avirulence gene complex with a section that is amplified and spread to different parts of the genome (Jiang *et al*., 2006). They suggest such a modular structure is consistent with a mechanism for generating genes with novel functions via shuffling and could be a novel mechanism for pathogens to rapidly adapt to environmental change.

It has been proposed that the displacement of the long-established A1 clonal lineage discussed in section 4a stemmed from a decline in its fitness and aggressiveness brought about by the accumulation of deleterious genetic mutations (Goodwin, 1997). It is commonly accepted that the sexual cycle and resultant genetic recombination prevents such damaging accumulations and moreover, generates novel combinations of genes that increases the potential of a population to adapt (Barton & Charlesworth, 1998). Increased aggressiveness is thus an oft-quoted predicted outcome of sexual recombination in *P. infestans*. Few studies on the inheritance of aggressiveness have however been reported. Aggressiveness comparisons of progeny of a cross between two potato aggressive isolates, one of which was also tomato aggressive, indicated a locus with a strong effect on tomato aggressiveness (Lee *et al*., 2002). Another study examined the sexual progeny of a cross between two aggressive lineages and showed that all were less aggressive than either parent and unable to generate a detectable field epidemic (Mayton *et al*., 2000). This does not mean the threat is not real, more that the factors influencing successful mating and generating aggressive progeny are unclear.

*Phytophthora* is not confined to sexual mechanisms to generate variation; a repertoire of alternatives is available (Brasier, 1992; Pipe *et al.,* 2000). Caten and Jinks (1968) for example, conducted a detailed study of variation in growth rate and sporulation amongst single asexually generated zoospore populations of three parental strains of the 'old' population. They revealed considerable variation amongst single zoospores and selection over several generations proved conclusively that the trait was heritable. In further tests these single zoospore progenies were tested on tubers and leaves and a great range of aggressiveness but

 

no changes in virulence phenotype observed (Caten, 1970). It should be noted however, than none were more aggressive than the original parent isolate. Caten (1970) suggested a form of cytoplasmic control and the same mechanism may be involved in cultivar-specific adaptation or 'training' of isolates for increased aggressiveness via repeated cycles of infection on a particular cultivar (e.g. Jinks and Grindle 1963; Hussain 2002) or the appearance of specific virulences within a clonal population (Pilet *et al.*, 2005; Goodwin *et al.,* 1995). Such epigenetic inheritance is the reversible but heritable change in phenotype (e.g. aggressiveness) that occurs in the absence of any change in genotype (e.g. mutation in the DNA sequence of that gene) and is likely to be a key research area in the future.

Genomics and functional genomics are illustrating the extent of gene duplication and the diverse battery of pathogenicity genes that is perhaps the signature of a successful pathogen. Understanding the way *P. infestans* is able to so readily adapt to its environment and to our control measures is a key step towards applying these theoretical advances into our understanding of the pathogen behaviour and ultimately disease management in the field.

 

# 8. Implications of increased aggressiveness on:

## *Effectiveness of current fungicide programmes*

Fungicides used in the UK for the control of late blight have all been evaluated against the current *P. infestans* population. There is no reason to expect that there will suddenly be a loss of control either in general or by specific fungicides, unless resistant strains arise unexpectedly to a new fungicide group (which would be a result of fungicide resistance not increased aggressiveness). In general, there is no evidence from trials in Europe or North America which suggests differences in the relative performance of different fungicides have resulted from population changes. However, the need to start fungicide programmes earlier and to use more applications at shorter intervals may reflect the greater aggressiveness of current pathogen populations. In this context, it would be informative to compare blight control in current and much earlier fungicide trials in relation to the number of fungicide sprays and the spray intervals required. Suitable results for season-long applications of fungicides, e.g. mancozeb or cymoxanil + mancozeb, on susceptible maincrop varieties are probably available from several sources (SAC, ADAS, AFBI).

In comparative fungicide trials in GB the efficacy of fungicides is evaluated using recent UK isolates (usually more than one) from blighted potato crops. However, it is not standard practice to ascertain the full phenotypic and genotypic characteristics of the isolates used.

In a computer simulation experiment in which isolate differences in latent period, lesion size and sporulation were taken into account, Kato *et al*. (1997) found that significantly more protectant fungicide (e.g. 25%) was likely to be required to control a foliar epidemic caused by the US-8 lineage compared with the old US-1 genotypes.

## *Accuracy of National List blight ratings*

Clearly, accurate National List (NL) resistance ratings are important to provide the industry with cultivar-specific information to inform blight control strategies. These values will however be affected by any changes in aggressiveness. A recent study (Hansen *et al.,* 2005) and the EU-funded Eucablight project (www.eucablight.org) advocate the evaluation of NL resistance ratings (on a 1-9 scale) by the inclusion of 'standard cultivars' in an international field trial network. In this way the scale can be derived from a comparison with the standards grown at the same site and compared with values obtained at other sites and in other countries. This process identified several discrepancies between the original and recently-derived values and highlights the need to keep the lists updated.

Furthermore, any changes in the rankings of standard cultivars may indicate a shift in population and this system will provide early warning of such changes. A marked failure of the cultivar Karnico, rated 8 for blight resistance, in a study in the Netherlands in which fluazinam dose was altered in line with published resistance figures (Wander *et al.,* 2006) illustrates the importance of accurate NL ratings. If increased aggressiveness is not cultivar-specific then the relative ranking of cultivars will be unaffected but control measures will nonetheless need to be tightened. This does however assume that the ratings are generated using isolates representative of the current population. Flier *et al.* (1998) demonstrated a

 

problem with the isolates used to generate the Dutch resistance ratings. Cultivar x isolate interactions are frequently reported so the accuracy of ratings will very likely be affected. The ratings can be incorporated into DSSs (this is common in some European countries but not in GB) and it is thus important that they are accurate. Updates of the national list blight resistance ratings for key commercial varieties is worthy of consideration.

## *Resistance breeding*

Breeding for resistance to late blight has now become a priority in most European countries as the disease has become more prominent. Although most breeders attempt to base their resistance on polygenic resistance, the inclusion of R-genes with large effect is difficult to avoid. Thus many new cultivars have high partial resistance in addition to one or more of the recognised R genes; the latter will give added protection if the complementary virulences are not present. Other promising clones show high resistance which, in the absence of genetic data or evidence of breakdown, is assumed to be polygenic. As there is evidence of specific aggressiveness of some pathogen genotypes, the breeder must expect that the pathogen might have at least some potential to adapt to a new clone.

It is thus essential that new clones be exposed to the most virulent and most aggressive genotypes of the pathogen which occur. In practice this is done by growing small plots in many locations in UK and abroad. Inoculation of field trials, if attempted, should be with an isolate which has shown complex virulence and compatibility with cultivars showing the highest field resistance. Alternatively, a mixture of such isolates from different sources may be used. In addition, whole plants or detached leaflets may be tested in the laboratory with a wider range of isolates including foreign ones to give a more complete assessment of likely stability of resistance. It would make sense if the same isolate(s) were used by breeders and for national listing. As the breeder's target is moving continuously, it is necessary to use a selection of the most recent isolates available as inocula (e.g. from current Fight Against Blight initiatives).

## *Reliability of blight risk forecasting models, i.e. effects of any changes (e.g. in temperature range, RH requirements) on epidemiological parameters*

An obvious means of optimising fungicide input is to incorporate cultivar resistance into the decision support system (DSS) models to allow an increase in spray interval or reduction in dose (Spits *et al*., 2005; Wander *et al*., 2006). However the model underlying the DSS must be robust and account for changes in the associated factors of pathogen aggressiveness and cultivar resistance. In predicting pathogen infection and spread the simulation models use pathogen and host parameters. Pathogen parameters are constants such as latent period and lesion growth rate and are based on the presumed worst-case scenario of an aggressive pathogen strain on a highly susceptible cultivar under optimal conditions. The host parameters are multiplication factors according to three resistance categories (e.g. Andrade-Piedra *et al.*, 2005). However the data behind these parameters need to be evaluated periodically. A model developed against a less aggressive pathogen population will obviously fail to accurately predict disease should aggressiveness increase. It is thus critical that the DSS systems are updated with accurate host resistance and pathogen aggressiveness data. The

 

minutes of the EU.NET.ICP meeting on DSS systems stressed the importance of accurate host resistance data and monitoring of the pathogen population (Kessel & Hansen, 2006).

There is evidence of disease development under conditions currently considered unsuitable for blight infection e.g. during non-Smith Periods (Shaw *et al.*, 2006). Very little is known about the growth and reproductive capacities of current strains of *P. infestans* over a range of temperatures and humidities. A pilot study for BPC indicated substantial variation in such capacities among a collection of 2004 isolates (Shaw *et al.*, 2006).

It is suggested that increases in aggressiveness will have little or no effect on chemical control other than an increase in the quantity of fungicide required to maintain a given level of control. However, the GB potato industry is currently under pressure to reduce, not increase, fungicide inputs. The assessment of resistance in National Listing programmes and breeding programmes needs to be based on the most virulent and aggressive strains of the pathogen. DSS models need to take account of any increases of aggressiveness (i.e. decrease in resistance) and changes in the ability of the pathogen to function in formerly suboptimal conditions. Such changes have not been examined.

# 9. Lessons for the GB industry from experiences in other countries

Members of the NorPhyt project conducted multi-site aggressiveness testing of a series of Nordic isolates with great attention to the standardisation of the laboratory protocols. Despite such rigour, marked site-specific differences were observed in the results, highlighting the challenge of such laboratory testing. However, the inoculation of field plots with isolates of high or low aggressiveness (as assessed on detached potato leaflets) indicated clear differences in disease severity and supported the general trends revealed by *in vitro* testing (Björn Andersson, Personal Communication, Swedish University of Agricultural Sciences, Uppsala; Hansen *et al.*, 2006). A range of aggressiveness was demonstrated during a presentation at the EU.NET.ICP meeting in Tallinn, but no clear differences between populations in Nordic states were evident (Hannukkala, A., personal communication, Agrifood Research Finland, Jokioinen). As aggressiveness is a trait routinely assessed by relatively few researchers, a decision was made not to include this data within the Eucablight pathogen database, but to record where it existed. In addition, aggressiveness tested by different methods across years and countries is a difficult trait to standardize for analysis. Of the 13,000 *P. infestans* isolates described within the Eucablight database, 1094 are known to have data relating to aggressiveness. Of these, 850 isolates tested in Poland over a 10 year period forms the most comprehensive data set.

Collaboration with Michigan State University and the USDA, has demonstrated the greater aggressiveness in the field of the US-8 clonal lineage compared with other current US *P. infestans* genotypes; direct comparison of US and UK genotypes has only been possible in the laboratory (Carlisle *et al.*, 2002; Young *et al.*, 2006), but did not indicate that US-8 is much more aggressive than current UK genotypes. In the US, severe epidemics of late blight on commercial potato and tomato have given rise to concern in recent years and these have been associated with new genotypes (Deahl, KL, USDA, personal communication); collaboration between the UK and US allows comparisons which can help understanding of the behaviour of both pathogen populations.

## *Are concerns about pathogen migration justified?*

Historically Europe has had a long period with only a single or very few, clonal lineage(s) (1845-1980s) followed by an increase in diversity in the early 1980s and a period of stabilisation up until 2004. A period of transition is now apparent with new lineages of A2 strains emerging (Shaw *et al*, 2006; Cooke *et al*., unpublished, BPC blight survey project). All the major population displacements have occurred as a result of immigrant populations rather than new types emerging as a result of mutation of the existing population. These migrations have all resulted from human intervention: humans are the most important vectors of *P. infestans* over long distances, through international trade in potatoes and tomatoes. As presented in this review, there is evidence that such displacements are a function of the new strains having an advantage over the existing ones. An ability to outcompete by increased aggressiveness, fitness or fungicide resistance are clear driving forces and all such traits will clearly impact on potato growers faced with a pathogen that is harder to control. Where

 

fungicide resistance (phenylamide resistance) has hitch-hiked on the back of increased aggressiveness, so that an aggressive, phenylamide-resistant population has displaced a less aggressive sensitive one (e.g. in Taiwan, Deahl *et al.,* 2002), major control problems result and fungicide resistance management is ineffective. South or Central America is the centre of *P. infestans* diversity and European populations are inevitably therefore less diverse. It is not possible to quantify but nonetheless there is a threat and the GB potato industry should remain vigilant and prevent the introduction of new strains.

 

# 10. Research needs (R&D gaps)

Recent BPC funded work has indicated that marked changes are occurring in GB *P. infestans* populations, in particular an increase in the frequency of the A2 type. Existing studies are devoted to monitoring such changes and assessing the risk of oospore formation. To complement this, an objective assessment of the impact of any changes is needed in order to provide relevant data to industry to optimise disease management practices. A series of interrelated research areas, each of which needs to relate to the current GB blight survey project (R274) and FAB activities, are highlighted below:

1. Improved understanding of the aggressiveness and fitness of current populations
   a) Screening of, representative isolates of the most prevalent GB *P. infestans* genotypes against leaves and tubers of a range of current cultivars in the laboratory to provide preliminary information concerning variation in aggressiveness. To include scoring of a limited number of key criteria such as infection efficiency and lesion expansion.
   b) Validation of results of 1a. under field conditions by examination of infection efficiency and spread of representative isolates from 1.a) in small 4-plant field plots of a range of cultivars.
   c) Provision of objective field data through an extension of the study to include larger scale field plots of a range of cultivars in which mixed inoculum is introduced and the aggressiveness of different genotypes is monitored by tracking using SSR markers. (Cooke *et al.,* SCRI, Shaw *et al.* Sárvári Research Trust and Cooke *et al.* AFBI have all been conducting such studies).
   d) Aggressiveness is a measure of short-term success within the polycyclic phase. It is also important to examine the real issue of parasitic fitness considering the survival from season to season. The over-wintering phase i.e. blight survival on tubers (seed, volunteers and in outgrade piles) to be examined as a continuation of the trials in 1c. Such studies would reveal whether seed of certain cultivars is more efficient at transmitting blight into the next season. Isolate tracking with SSR markers is fundamental to this approach.
   e) Regular review of the above (a-d) activities to ensure the isolates and cvs tested are still appropriate and ensure results generated have direct and immediate relevance to industry. This requires co-ordination with the activities of the survey project (R274). For example, if mixed mating type sites are identified in the BPC survey project isolates could be selected and compared to clonal types to examine any aggressiveness changes.
   f) Periodic assessment of aggressiveness changes should be considered in the longer term to identify any trends.

2. Reliability of Decision Support Systems
   a) Complementation of the above studies of aggressiveness with studies of the temperature/leaf surface wetness/relative humidity interactions of the same isolates to determine the extent of variation in isolates' interactions with the physical environment. Findings are essential for any refinement of DSS models which would be necessary if extreme ecotypes are identified.

 

b) Evaluation of appropriate DSS systems with representative isolates of the contemporary population to examine whether the criteria remain appropriate or have changed due to changes in population aggressiveness.

The above studies would help identify the extent to which *P. infestans* is operating at temperatures and relative humidities previously considered to be sub-optimal.

3. Host Resistance
   a) A review is urgently required to determine whether the GB approach to control (relying very heavily on fungicide control) needs a major re-think to ensure effective control where the population is substantially more aggressive. It is anticipated that in these circumstances there will be a greater need to exploit cultivar resistance and integrate this with fungicide usage. Results from fungicide evaluation trials carried out with the most recent isolates (see 4b) will partly determine to what extent greater use of cultivar resistance will be required in future.
   b) Related to this is the need to ensure the resistance ratings of commercially important cultivars are kept up to date. Following on from EUCABLIGHT recommendations, National List scores for new and old cultivars with moderate to high foliage and/or tuber blight resistance should be reviewed and updated with new GB data and with pan-European data from field trials using local isolates/populations and the agreed European standard cultivars. National List scores for varieties which, it is generally agreed are less resistant than previously, due to evolution of the pathogen need to be adjusted at the earliest opportunity. This information should link to the BPC funded Independent Variety Trialling for late blight. More accurate data on resistance and its stability should encourage the industry to reduce the fungicide load by deploying resistance.
   c) There appears to be a conflict between some researchers who wish to use a single complex virulence race of *P. infestans* in cultivar resistance screening and other researchers who advocate the use of many, very recent isolates. It has been demonstrated that the results obtained can be substantially affected. The discrepancy between these two approaches needs to be resolved so that cultivar resistance ratings are robust.
   d) Quantification of the impact of cultivar x isolate interactions (for foliar and tuber blight) on the efficacy of cultivar resistance. The relative occurrence of such interactions and their magnitude need to be quantified to allow their importance in blight management to be properly understood.

4. Fungicide Efficacy
   a) A comparison of blight control in current, and former, fungicide trials, in relation to the number of fungicide sprays and the spray intervals required, would identify any trend for increased aggressiveness over decades. This would require results from various research centres (SAC, ADAS, AFBI) to be combined. Suitable results would be season-long applications of fungicides, e.g. mancozeb or cymoxanil + mancozeb, on susceptible maincrop varieties.
   b) Independent assessment of the effectiveness of current fungicides against new genotypes of the pathogen is required. It is recommended that the *P. infestans* isolates used in fungicide trials, including product registration trials, are genotyped so that it is known which "population" of *P. infestans* was tested. Procedures should be set up

 

(with PSD approval) so that only appropriate isolates from the new populations are used.

5. Role of alternative inoculum sources in GB *P. infestans* populations.
   a) The risks of importation of *P. infestans* strains with increased aggressiveness on seed and ware potatoes should be assessed.
   b) An examination of prevalence and aggressiveness of blight from other solanaceous plants (e.g. nightshade weeds and the PCN trap crop, *S. sisymbriifolium*). This could perhaps tie in with current PCN trap crop projects.
   c) As the FAB campaigns reduce the inoculum from dumps and volunteers, the contribution of inoculum from unprotected potato and tomato crops in gardens and allotments will become more apparent. Both mating types have been regularly detected in such situations which may be an important source of recombinant strains of late blight. Monitoring of these populations to determine their importance should become a component of the FAB campaign in the future.

6. Identification and exploitation of a panel of *P. infestans* isolates reflecting the range of aggressiveness observed in GB.
   a) A panel of isolates representative of current populations should be compiled and recommended for use in national list and IVT scoring, fungicide evaluation and breeding material screening. This will ensure data from such sources is relevant and representative.

 

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