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## DOCTOR OF PHILOSOPHY

### Late blight (*Phytophthora infestans*) on tomato: evaluation of pathogen population structure in Britain and development of resistant tomato cultivars for growing outdoors

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**Late blight (*Phytophthora infestans*) on tomato: evaluation of pathogen population structure in Britain and development of resistant tomato cultivars for growing outdoors**

A dissertation presented by

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Supervised by Dr. Katherine Steele

In partial fulfilment of the requirements of the degree of

**PhD Plant Pathology**

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## List of Abbreviations Used

<b>Abbreviation</b>	<b>Meaning</b>
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
AVRDC	Asian Vegetable Research and Development Centre
BP	Base Pair(s)
CAPS	Cleaved Amplified Polymorphic Sequence
cM	Centimorgan(s)
DL	Detached Leaflet
DS	Disease Severity
FDR	False Detection Rate
HR	Hypersensitive Response
IE	Infection Efficiency
MtDNA	Mitochondrial DNA
NB-LRR	Nucleotide Binding Leucine Rich Repeat
NC(SU)	North Carolina (State University)
NIL	Near-Inbred Line
PAMP	Pathogen Associated Molecular Pattern
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
SAUDPC	Standardised Area Under Disease Progress Curve
SCAR	Sequence Characterised Amplified Region
SI	Sporulation Intensity
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TMV	Tobacco Mosaic Virus

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- Kitchen Garden Magazine: [www.kitchengarden.co.uk](http://www.kitchengarden.co.uk)
- Soil Association: [www.soilassociation.org](http://www.soilassociation.org)
- Grow it Yourself International: [www.giyinternational.org](http://www.giyinternational.org)
- RHS Wisley: [www.rhs.org.uk](http://www.rhs.org.uk)
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## Abstract

*Phytophthora infestans* is an Oomycete plant pathogen, and causes late blight disease on tomatoes and potatoes. This is arguably the most serious disease of these crops in temperate climates such as Britain's. An SSR genotyping study was undertaken to assess the extent to which *P. infestans* populations on tomato and potato in Britain are host-specialised. No evidence of host specialisation was found, although very high levels of genetic diversity were found in the *P. infestans* populations sampled from gardens and allotments, suggesting that these settings could be a source of new *P. infestans* genotypes, possibly arising from sexual recombination. Additionally, field trials and associated marker-assisted selection work were carried out in conjunction with Burpee Europe Ltd., Pro-Veg Seeds Ltd., and the Sarvari Research Trust, with the aim of developing blight-resistant tomato cultivars for commercial release. A wide range of germplasm was screened, identifying promising material for breeding work. A line developed in this project, carrying the *Ph-2* and *Ph-3* genes for *P. infestans* resistance, has been released by Burpee Europe Ltd. under the name "Crimson Crush". Additionally, detached leaflet studies were conducted to investigate how different resistance genes in combination interact with common *P. infestans* isolates. Findings included an indication of some residual effect of the "defeated" *Ph-1* gene, the potential of some aggressive *P. infestans* isolates recovered from British gardens to overcome combined *Ph-2* and *Ph-3* resistance, and an insight into how the *Ph-* genes affect components of resistance (i.e., infection efficiency, latent period, rate of lesion expansion, and sporulation intensity), and may be complementary to each other. Finally, a genetic mapping study was carried out to investigate the genetic basis of resistance seen in tomato cultivar Koralik. Resistance QTLs were not reliably identified in Koralik, but the study did indicate the presence of resistance QTLs on Chromosomes 4 and 7 of the other parent in the cross, NC2-CELBR. Potentially useful QTLs affecting soluble solids content of fruit were identified in Koralik. Taken as a whole, the findings of these studies indicated that novel tomato cultivars with *P. infestans* resistance from a broad range of genes are needed to combat the threat from a highly diverse and evolving *P. infestans* population. The breeding and mapping work undertaken in this project makes some contribution to addressing this challenge, although further work is needed to fully capitalise on this.

# 1 Literature Review

## 1.1 Biology of *Phytophthora infestans*

### 1.1.1 *P. infestans* life cycle

Under natural (and agricultural or horticultural) conditions, *P. infestans* is an obligate hemibiotrophic pathogen (Fry 2008b), requiring living tissue of a suitable host species for germination of spores. Sporangia landing on host plants in warmer conditions may germinate directly, via a germ tube which grows into the host tissue through a stoma or weak spot in the cuticle (Fry 2008b). Alternatively, in the presence of sufficient liquid water and low temperatures, sporangia may release zoospores, which possess flagellae and are able to swim to the most suitable infection sites, causing rapid infection (Walker and van West 2007). Zoospore release is more common at temperatures around 10-15°C (Melhus 1915) [cited in Fry (2008b)]. Direct germination tends to predominate at higher temperatures (Fry 2008b), up to 32.5°C, where viability of *P. infestans* begins to be compromised (Kable and Mackenzie 1980). Up to 300,000 sporangia per day can be produced by a single lesion (on potato), which facilitates the rapid spread of the disease (Govers 2005). Spore production has been observed to be suppressed by continuous illumination (Cohen *et al.* 1975), *et al* which may be a mechanism to ensure spore production is highest at times of low insolation and corresponding high humidity and low temperature, when spore viability is greatest (Fry 2008b).

Whilst oomycetes such as *P. infestans* appear fungus-like, their biochemistry is in fact rather different. They lack the chitinous cell walls typical of fungi, and instead have cell walls composed of (1-3) and (1-6)  $\beta$ -D-glucans and cellulose (Bartnicki-Garcia 1968). This fact, coupled with the first genetic studies, led to the Oomycetes being integrated into the distinct kingdom Stramenopiles (Leipe *et al.* 1996). However, owing to their fungus-like morphology and life-history, their study is generally considered to fall within the field of mycology, and they are frequently (albeit incorrectly) referred to as fungi.

*P. infestans* is capable of sexual and asexual reproduction. For sexual reproduction (and genetic recombination) A1 and A2 mating type genotypes must come into contact. In many areas with or without both mating types present, including Great Britain, reproduction is mostly or entirely asexual (Cooke *et al.* 2014; Collins, 2013; Lees *et al.* 2012). In these situations, recurrent clonal lineages dominate in *P. infestans* populations, with little or no genetic change from parent to offspring generations. Accumulation of mutations within clonal lineages leads to intra-lineage variation (Cooke *et al.* 2014). Novel clonal lineages may arise in agricultural systems through

more extensive mutation, mitotic recombination, sexual recombination, or migration from *P. infestans* diversity hotspots such as South America (Goodwin *et al.* 1994).

### 1.1.2 *P. infestans* growth in vitro

*P. infestans* is easily grown on a variety of artificial media, including “V8” vegetable juice agar, rye agar (Fry 2008a), entirely synthetic minimal medium (Hall 1959) as well artificial media prepared from agar or gelatine and various pulses or vegetables (Goth 1981; Sopee *et al.* 2012). Rye agar is the standard medium in many laboratories. The pathogenic fitness of *P. infestans* frequently declines with prolonged storage on artificial media (Caten and Jinks 1968). In order to avoid this problem, *P. infestans* may be stored on potato tubers or potato or tomato leaflets. Unfortunately, this is highly labour intensive as isolates must be transferred to fresh leaflets or tubers at intervals of 7-10 days, depending on ambient temperature.

### 1.1.3 The *P. infestans* genome

Owing to the great crop losses caused by *P. infestans*, its study has attracted considerable interest, with the genome having been sequenced in full (Haas *et al.* 2009). Compared to other oomycetes, *P. infestans* has a large genome, of around 240Mb. Other *Phytophthora* spp. typically have genomes of less than 100Mb. Most of the additional genetic material in *P. infestans* is comprised of repetitive DNA, which makes up approximately 74% of the genome. 17,797 coding genes were identified, generally located in blocks separated by large stretches of non-coding DNA (Haas *et al.* 2009).

Genetic diversity in *P. infestans* populations is high in many regions of the world; for example Flier *et al.* (2003) performed AFLP analysis on 170 Mexican isolates of *P. infestans* and found 158 distinct genotypes. The number of genotypes detected can vary considerably between different analysis methods. For example, Knapova and Gisi (2002) noted that when the same set of *P. infestans* isolates were analysed using SSR markers and AFLP fingerprints, 26 and 40 different genotypes, respectively, were distinguished. Whilst AFLP analysis may provide a greater ability to distinguish between isolates, it may not be as reproducible between laboratories as SSR marker analysis (Jones *et al.* 1997), and a standard set of 12 SSR markers has been adopted by Euroblight for inter-lab comparison on *P. infestans* genotype (Li *et al.* 2013). Marker systems for studying *P. infestans* are discussed more extensively in **Chapter 3**.

#### 1.1.4 Origin and recent migrations of *P. infestans*

*P. infestans* is thought to have originated in Mexico and spread to the South American Andes early in its evolution (Goss *et al.* 2014). It remained within this range until the North American and European potato blight epidemics of the 1840s (Bourke 1964), which appear to have been caused by the introduction of several closely related *P. infestans* isolates (Martin *et al.* 2014).

These isolates belong to the HERB-1 clonal lineage, named on account of its rediscovery in herbarium specimens collected during the second half of the 19<sup>th</sup> century (Yoshida *et al.* 2013). *P. infestans* HERB-1 was displaced by the closely related US-1 lineage (Yoshida *et al.* 2013), which was subsequently the only clonal lineage detected outside South America and Mexico (Goodwin *et al.* 1994b) and showed only minor genetic variation (Martin *et al.* 2014) until the next migration event in the late 1970s, which introduced new genotypes (including those with A2 mating types) from Mexico to Europe and subsequently to the rest of the world (Hohl and Iselin 1984; Niederhauser 1991; Shaw *et al.* 1985; Spielman *et al.* 1991). With the migration of A2 mating types in the late 1970s came the potential for sexual recombination and the rapid genetic diversification of the global *P. infestans* population. Thus, since the early 1980s, *P. infestans* populations in most European countries have consisted of multiple unique genotypes and recurrent clonal lineages (Euroblight 2014).

#### 1.1.5 Host specialisation by *P. infestans*

*P. infestans* is capable of infecting several species of Solanaceae worldwide, with *Solanum* species being the most frequent hosts (Nelson 2008). Of the species susceptible to *P. infestans*, potato and tomato are the two most economically important and widespread. In many global regions where multiple *P. infestans* genotypes and multiple host species are present, some degree of host-specialisation has been observed, both in terms of the frequency of recurrent clonal lineages on each host, and in terms of within-lineage genotypic and phenotypic variation. For example, multiple studies in the USA have demonstrated that the US-8 lineage is common on potato, but very seldom infects tomato (Danies *et al.* 2012; Peters *et al.* 2014; Wangsomboondee *et al.* 2002). In studies where inoculations onto tomato and potato leaflets have been carried out, isolates are often found to be more pathogenic on one host than another. This specialisation may correspond to the *P. infestans* clonal lineage (Danies *et al.* 2012) or to the host species from which the isolate was originally collected (Garry *et al.* 2005; Knapova and Gisi 2002; Lebreton *et al.* 1999; Oyarzun *et al.* 1998; Vega-Sanchez *et al.* 2007). A number of studies have been carried out in different regions investigating the distribution of *P. infestans* genotypes on different hosts, and their phenotypic adaptation to them (**Table 1.1**). Probably due to the lack of an outdoor tomato



industry in Great Britain, the question of what, if any, host specialisation takes place in this country does not appear to have been addressed in the literature.

**Table 1.1: Previous studies which have investigated host specialisation by *P. infestans*.**

Location and Years	Findings	Study
Peru 1997-2000	<p><b>Clonal lineage:</b> Strong apparent association between potato host and EC-1 and PE-3 lineages, and between wild tomato spp. and PE-7 (although the host populations were geographically separated.)</p> <p><b>Aggressiveness:</b> EC-1 isolates from <i>S. tuberosum</i> were more aggressive on their host of origin than on <i>S. caripense</i>, although EC-1 isolates from <i>S. caripense</i> were similarly aggressive on both hosts. US-1 isolates from <i>S. caripense</i> were less aggressive on <i>S. tuberosum</i>, however.</p>	Garry <i>et al.</i> (2005)
Ecuador 1993-1995	<p><b>Clonal lineage:</b> All 60 Potato-derived isolates were EC-1. Of 59 tomato isolates, 60 were US-1 (and 1 EC-1).</p> <p><b>Virulence:</b> Potato isolates were able to overcome between 6 and 11 potato R-genes, but frequently few or no tomato <i>Ph</i>-genes. Similarly, most tomato isolates overcame all tomato <i>Ph</i>-genes (<i>Ph-1</i>, <i>Ph-2</i> and an additional putative resistance gene identified in the study) but few or no potato R-genes.</p> <p><b>Aggressiveness:</b> Average lesion diameter on alternative host was generally around half that of host of origin.</p>	Oyarzun <i>et al.</i> (1998)
Brazil [years unavailable]	<p><b>Clonal lineage:</b> Most tomato isolates were US-1, all potato isolates were BR-1.</p>	Brommonschenkel (1988)
S and SE Brazil 1998-2000	<p><b>Mating type:</b> Of 267 tomato isolates, 100% were A1. Of 184 potato isolates, 82% were A2.</p> <p><b>Clonal lineage:</b> Of 54 tomato isolates tested, 53 were US-1 or variants thereof, whereas 34 out of 39 potato isolates were BR-1 or BR1.1 (with the remainder being US-1 variants).</p>	Reis <i>et al.</i> (2003)
USA, Mexico, Canada and Netherlands 1980s- 1990s	<p><b>Aggressiveness:</b> two classes were observed: isolates that were equally aggressive (in terms of rate of lesion expansion and spore production) on tomato and potato and isolates that were less aggressive on tomato than potato.</p>	Legard, Lee & Fry (1995)

Location and Years	Findings	Study
N Carolina, USA. 1993-1998	<p><b>Mating Type:</b> All isolates were A1.</p> <p><b>Clonal lineage:</b> Of 157 potato derived isolates, 154 (98%) were of the US-8 clonal lineage, whereas of 93 tomato-derived isolates, 53 (57%) were US-7, 20 (22%) were US-18, 15 (16%) were US-8, and 5 (5%) were US-19. However, the authors note that in North Carolina, potato and tomato crops are geographically separated, and the initial late-blight infection is probably from different sources.</p>	Wangsom-boondee <i>et al.</i> (2002)
USA and Canada, 2002-2008	<p><b>Clonal Lineage:</b> Of 40 isolates collected from tomato, 20 (50%) were US-22, 14 (35%) were US 20, and 6 (15%) were US-21. US-21 was observed to be non-pathogenic on potato.</p>	Fry <i>et al.</i> (2013)
USA and Canada, 2009-2011	<p><b>Clonal Lineage:</b> Of 142 isolates from tomato, 86 (61%) were US-22, and 54 (38%) were US-23, with two (1%) US-24 isolates. Of 110 isolates from potato, 57 (52%) were US-24, 25 (23%) were US-8, 15 (14%) were US-22, and 13 (12%) were US-23.</p> <p><b>Aggressiveness:</b> Clonal lineages US-8 and US-24 were significantly more aggressive on potato than tomato in terms of lesion size and sporulation intensity at 6 days post inoculation (DPI). Lineages US-22 and US-23 were significantly more aggressive on tomato in terms of sporulation intensity, and there was no significant difference in lesion size between the two hosts at 6 DPI for these lineages.</p>	Danies <i>et al.</i> (2012)

Location and Years	Findings	Study
Canada 2011	<p><b>Mating Type:</b> Of 20 tomato derived isolates, 9 (45%) were A1 and 11 (55%) A2. Of 109 potato derived isolates, 58 (53%) were A1, 51 (47%) were A2 (not significantly different).</p> <p><b>Clonal Lineage:</b> Of 20 tomato derived isolates, 10 (50%) were US-22, 4 (20%) were CA-10, and the remaining six isolates were CA-9, CA-11, US-11 and US-23. Of 118 potato-derived isolates, 51 (47%) were US-8, 41 (38%) were US-24, and the remainder were US-11 and US-23. Simpson's diversity index was similar for both (0.32 and 0.33 respectively), but the distribution of isolates was significantly different. Samples were geographically clumped, and in many cases samples from the same location were all of the same lineage.</p>	Peters <i>et al.</i> (2014)
Uganda and Kenya 1995, 1997	<p><b>Mating Type:</b> All isolates were A1</p> <p><b>Clonal lineage:</b> All isolates were US-1, although in all cases tested, <i>Gpi</i> allozyme genotype of potato isolates was 86/100, whereas tomato isolates were all 100/100 (US-1 variant US-1.7).</p> <p><b>Aggressiveness:</b> Most tomato isolates grew poorly on potato tuber slices during isolation (<i>ad hoc</i> observation), and a subset of 12 isolates that were virulent on both hosts showed much greater aggressiveness on their host of origin.</p>	Vega-Sanchez <i>et al.</i> (2007)
India 2010-2012	<p><b>Mating Type:</b> All 63 potato isolates and 94 tomato isolates were A2.</p> <p><b>Clonal Lineage:</b> All isolates had the RFLP and MtDNA genotype of the 13_A2 clonal lineage. Analysis with 12 SSR markers was performed on 27 potato isolates and 18 tomato isolates. The SSR analysis confirmed that all isolates were 13_A2, although minor variation was detected at two more variable loci. All potato isolates were variant 13_A2_3. The tomato isolates included 13_A2_3 and three other variants.</p> <p><b>Aggressiveness:</b> All isolates were equally aggressive on detached susceptible tomato and potato leaflets.</p>	Chowdappa <i>et al.</i> (2013)

Location and Years	Findings	Study
France Pre-1992, 1996	<p><b>Mating Type:</b> The distribution of mating types was similar on both hosts (on tomato, 30 of 36 isolates (83%) were A1, with the remainder A2, whilst on potato, 37 of 40 (93%) were A1, with the remainder A2.</p> <p><b>Clonal Lineage:</b> The potato sample was dominated by the FR-01 lineage, in contrast to tomato. Of the 37 isolates from potato, 28 (70%) were FR-01, five (13%) were FR-04, and the remainder of the sample was made up of four other lineages. Of 34 tomato samples genotyped, 16 (47%) were FR=01. The remainder of the tomato sample was made up of seven FR-07 isolates (19%) and five further lineages at frequencies below 10%. Genotypic diversity was greater on potato (<math>H_s = 1.08</math>) compared to tomato (<math>H_s = 1.41</math>).</p>	Lebreton and Andrivon (1998)
France 1995-1996	<p><b>Infection Efficiency:</b> Infection efficiency on potato was significantly higher for isolates collected from potato (at 92%) than from tomato (at 79%). No difference in infection efficiency dependant on host of origin was detected when tomato leaves were inoculated.</p> <p><b>Lesion Expansion Rate:</b> Tomato-derived isolates produced faster-expanding lesions than those from potato on both hosts during most stages of the experiment.</p> <p><b>Sporulation Intensity:</b> Potato-derived isolates sporulated more abundantly than those from tomato when inoculated onto potato leaflets (no inoculations were made onto tomato for this experiment).</p> <p><b>Field Fitness:</b> When two replicate field plantings of susceptible potato plants were point-inoculated with a potato-derived and tomato-derived isolate, only the potato-derived isolate spread to non-inoculated plants in either replicate.</p>	Lebreton <i>et al.</i> (1999)

Location and Years	Findings	Study
France and Switzerland 1996-1997	<p><b>Mating type:</b> Of 134 potato isolates, 4% were A2, compared with 50% of 42 tomato derived isolates.</p> <p><b>AFLP:</b> AFLP genotypes formed seven clusters. Tomato isolates belonged to only three of these, whereas potato isolates were included in all seven.</p> <p><b>MtDNA:</b> No obvious host association; 93% of isolates were Ia.</p> <p><b>Microsatellite Genotype:</b> Higher diversity and higher proportion of heterozygotes on tomato than potato.</p> <p><b>Virulence:</b> Potato-derived isolates were frequently of complex races, infecting an average of 7.6 R-gene differentials, while those from tomato infected on average only 3.4.</p> <p><b>Aggressiveness:</b> Potato-derived isolates were significantly less aggressive on tomato than were tomato-derived isolates, although the tomato-derived isolates were equally aggressive to both hosts.</p>	Knapova & Gisi (2002)
Moscow Region, Russia 2008-2011	<p><b>Mating Type:</b> All 14 tomato isolates were A2. Of 24 potato isolates, 15 (63%) were A1 and 9 (37%) were A2.</p> <p><b>Clonal Lineage:</b> There was little allozyme variation. All nine tested tomato isolates had the same RFLP genotype. The 20 tested potato isolates were divided into 11 RFLP lineages. The 12 SSR markers distinguished two tomato genotypes amongst the 14 tested isolates and 16 genotypes amongst the 24 potato isolates. Explicit comparisons were not made, but the authors believe the tomato and potato populations to be genetically distinct.</p>	Statsyuk <i>et al.</i> (2014)

## 1.2 Biology and cultivation of Tomato (*Solanum lycopersicum*)

### 1.2.1 Agricultural importance of tomato

Global production of tomatoes in 2013 was approximately 164 million tonnes (FAOSTAT 2013), making them the most important vegetable crop (in terms of weight produced) after potatoes worldwide. Two broad groups of tomato cultivars with differing characteristics exist – those primarily intended for consumption in their raw, fresh state, and processing tomatoes, which typically have higher dry matter content, contain less seed gel, and are better suited for canning and use in soups, sauces, and other cooked foods (Salunkhe and Kadam 1998). A further distinction exists between cultivars which ripen fruit sequentially, versus those on which all fruit ripen simultaneously. Most processing tomato cultivars are bred to ripen all fruit simultaneously to facilitate destructive mechanical harvesting (Salunkhe and Kadam 1998). Simultaneous ripening of fruit may also be advantageous for commercial growers of fresh tomato, but cultivars bred primarily for amateur gardeners or small-scale commercial growers typically ripen fruit sequentially to allow picking over a longer season. Most of the world's processing tomato crop is cultivated outdoors in warmer, drier regions, with California, for example, accounting for 35% of the world's production (Hartz *et al.* 2008).

### 1.2.2 Impact of *P. infestans* on tomato cultivation

*P. infestans* spores require liquid water on foliage, or 100% relative humidity, to infect plants, and the pathogen grows and reproduces fastest at temperatures below about 25 °C (Fry 2008b), so does not generally infect plants grown in greenhouses. In most hotter, drier regions such as California, where tomatoes are grown outdoors, they are vulnerable to *P. infestans* infection at wetter times of year, and so are protected with fungicides (although *P. infestans* is an oomycete, protectant chemicals are still classed as fungicides) at these times (Hartz *et al.* 2008). In cooler, wetter areas, such as Northern Europe, commercial outdoor tomato production is rare, largely because of the threat from *P. infestans*. In Britain, commercial tomato crops were grown outdoors in the Channel Islands and the South of England in the 19<sup>th</sup> and 20<sup>th</sup> centuries and the practice continued on a limited scale in Jersey until at least 2005, when the last grower ceased outdoor production owing largely to the risk of late-blight infection and lack of availability of listed fungicides with which to prevent it (Le Maistre 2006). Today, all significant commercial tomato production in Britain takes place in greenhouses, which produce tomatoes for fresh consumption (Heuvelink 2005). Therefore, there is little demand from commercial growers in Britain for blight-resistant tomatoes. However, amongst amateur gardeners there is a large potential market for blight-resistant outdoor cultivars, as tomatoes are reported as being the most popular crop amongst amateur gardeners (Staub 2010), so developing blight-resistant cultivars is of great

interest to breeders catering to the amateur market. The Horticultural Trades Association reported that on average, British households spent about £130 on their gardens per year in 2013 (HTA 2014) and in 2010 the total UK gardening market was worth £4.6 bn (HTA 2011).

### 1.2.3 *P. infestans* control strategies

The main crop species affected by *P. infestans* are potatoes and tomatoes. There are four effective control strategies:

#### 1.2.3.1 *Physical protection*

As previously noted, physical protection from rain, dew, and to a lesser extent, spores, can be achieved by growing in glasshouses or tunnels, and is highly effective (Collins 2013; Nelson 2008). This is often the preferred option for amateur gardeners, but erecting a greenhouse or polytunnel is capital intensive, probably necessitates ground (as opposed to balcony or patio) space, and is often forbidden on rented allotments.

#### 1.2.3.2 *Protective sprays*

A number of protectant fungicides and organic preparations are, or have been, available for particular applications:

**Organic preparations** consisting of plant extracts or bacterial cultures. None are curative, but preventative effectiveness ranges from slight to fairly good. For example, Gevens (2013) reported reductions in disease severity of approximately 80% by several such products applied before inoculation, compared with no treatment.

**Copper-based fungicides** are effective preventatives, although not curatives. However, they pose environmental toxicity problems such as toxicity to soil organisms following prolonged use and resultant copper accumulation (Wightwick *et al.* 2008; Komarek *et al.* 2010), and modest human toxicity (Fishel 2005) as such their use is not encouraged (Pears 2005). They are permitted under organic standards (Organic Farmers & Growers 2015) and are available to amateur gardeners (Gevens 2013).

**Synthetic fungicides** such as metalaxyl, mancozeb, fluazinam and carbamates are often highly effective, with some having curative, as well as preventative, action. However, following the widespread use of many of these, particularly metalaxyl, resistance to them does occur in *P. infestans* populations (Day *et al.* 2004). None of these synthetic fungicides are available to gardeners for protection of fruit and vegetable crops. Many amateur gardeners do not wish to use copper or synthetic fungicides (the latter being unavailable to them in Britain today (RHS 2014)), whilst more are willing to use organic preparations which are less effective and may be expensive



so in many cases are not an acceptable option. Finally, most sprays, and particularly those available to gardeners, must be applied regularly before the crop is infected in order to be most effective (Gevens 2013) and amateur gardeners may be less likely to use them in this way.

### 1.2.3.3 Genetic resistance

In many respects, genetic resistance is the ideal option for amateur gardeners (and indeed commercial growers), as it requires no special capital investment in greenhouses, presents little or no risk to the environment or grower's safety (which fungicides and even some organic preparations may do) and it can be highly effective, particularly when compared to post-infection treatment with the fungicidal products available to amateur gardeners. A number of resistant cultivars (for example, Mountain Magic, Mountain Merit, Iron Lady and Defiant) are available worldwide, although due to licencing and distribution agreement limitations, not all are available in Britain. Therefore, there is a strong imperative on breeders and seed companies to develop blight resistant cultivars adapted to British conditions.

### 1.2.3.4 Good biosecurity measures

Measures to minimise the availability of inoculum at the beginning of each season should be practiced in addition to any other measures used to control blight. Ensuring that viable potato tubers are not left in the environment over winter (in the case of volunteer potatoes remaining the ground these should be removed or killed with herbicide at the first opportunity in the spring) helps to prevent carry-over of *P. infestans* mycelium in the living potato tissue from one year to the next (Schumann and D'Arcy 2005). For the same reason, only disease-free potato seed tubers should be used. *P. infestans* infection of tomato transplants stocked in nurseries would seem less likely than of potato tubers. However, the spread of the US-22 strain across much of the eastern USA in 2009 via this route showed that nursery stock could indeed be an important inoculum source (Fry *et al.* 2013), so gardeners should also be vigilant against buying infected tomato transplants. Biosecurity measures such as these can prevent initial infection from within a gardener's own premises or allotment, and are generally regarded as good practice to prevent the transmission of the disease to other growers' crops. For this reason, in the event of a disease outbreak, infected plants should also be destroyed if they begin to sporulate heavily (Schumann and D'Arcy 2005). Unfortunately, owing to the fact that *P. infestans* spores can survive for several hours under favourable weather conditions (Minogue and Fry 1981) and aerial spores are detectable at least 500 m from their source (Aylor *et al.* 2011) and may disperse several kilometres (Fry 1998), good biosecurity alone is unlikely to reliably protect crops in most settings.

## 1.3 Genetic resistance to plant diseases

### 1.3.1 Introduction to quantitative and qualitative traits

Phenotypic traits possessed by organisms may be qualitative and clearly fall into one of a limited number of discrete classes (a familiar example being human blood group) or quantitative, grading continuously from one extreme trait value to another (for example, human height at maturity). The quantitative versus qualitative nature of a trait is partly due to the extent to which the trait is susceptible to influence by environmental factors, and partly due to the nature of the genetic control over the trait. For example, a human's height at maturity is strongly influenced by environmental factors such as their nutritional status and physical activity level during development (Silventoinen 2003). In contrast, human blood group appears to be under strict genetic control, with an individual's environment having no effect (Yoshida 1981). It is intuitive that some traits should be more strongly affected by environmental factors (particularly during development) than others. In addition to different sensitivity to environmental factors, the nature of the genetic control over a trait also determines whether it will be qualitatively or quantitatively expressed. Generally, qualitative traits are controlled by just one or a few genes, whilst quantitative traits are frequently controlled by a combination of many different genes. In the case of "major genes" controlling a qualitative trait, substituting an allele (or all copies of an allele in the case of a dominant gene) will result in a clearly different phenotype. In contrast, in the case of "minor genes" controlling a quantitative trait, substituting an allele (or alleles) will have a much smaller effect on the individual's phenotype. The small effect of a single allele change may be obscured by "noise" variability in the trait, caused by environmental effects or measurement errors. These minor genes controlling continuously expressed traits are known as Quantitative Trait Loci (QTLs). In truth, QTLs are genes which segregate and are inherited in the same (Mendelian) way as any major gene, but the influence of environmental effects and limitations of measurement mean that their small individual effects grade into a continuous spectrum in practice (Young 1996).

In cultivated plants, many horticulturally relevant traits such as crop yield or time to maturity are quantitative, as opposed to qualitative (Tanksley 1993). Such traits are heavily influenced by environmental variation; for example, the effect of soil nutrient and moisture status on crop yield is easily appreciated, as is the effect of weather and climate on time to crop maturity. Major genes with large effects may affect these mainly quantitative traits (for example, the large gains in wheat yield that resulted from the introduction of major genes for dwarfing (Flintham *et al.* 1997)), but such traits are generally controlled by multiple QTLs.

### 1.3.2 Qualitative and quantitative disease resistance

Literature often distinguishes between “qualitative” or “vertical” resistance, and “quantitative” or “horizontal” resistance (Niks *et al.* 2011; Young 1996). The former provides very high levels of disease resistance, often through a hypersensitive response (HR). Here, cells rapidly die in the vicinity of an infection, preventing it spreading more than a few millimetres. This makes plants functionally immune to the disease (Fry 2008b; Niks *et al.* 2011). The *P. infestans* resistance R genes in potato (Vleeshouwers *et al.* 2000) and the *Ph-2* and *Ph-3* genes in tomato (Chunwongse *et al.* 2002; Turkensteen 1973) are generally considered to be qualitative resistance genes. Qualitative resistance genes (conferring immunity) are by definition major genes.

In contrast, quantitative/horizontal resistance genes typically do not provide immunity, but rather slow the spread of disease through plant tissue and reduce the rate of spore production. Quantitative resistance genes are often minor genes (QTLs) although this is necessarily always the case; a quantitative resistance gene with a large, easily observable effect may be considered to be a major gene. Example of such a genes would be the *Pi35(t)* gene conferring quantitative resistance to rice leaf blast (Nguyen *et al.* 2006) and the *Ph-2* gene conferring *P. infestans* resistance in tomato (Moreau *et al.* 1998) Examples of quantitative disease resistance conferred by multiple QTLs include 11 QTLs controlling *P. infestans* resistance in a potato mapping population (Leonards-Schippers *et al.* 1994), and 10 QTLs controlling resistance to *Magnaporthe grisea* in rice (Wang *et al.* 1994). Multi-gene quantitative disease resistance the advantage of being theoretically more durable than major-gene qualitative resistance, owing to the fact that a single mutation in the pathogen is less likely to overcome multiple QTLs than a single major gene. This has been borne out in the case of the multiple QTL mediated resistance to *Magnaporthe grisea* (rice blast) identified by Wang *et al.* (1994), which has proved durable despite widespread cultivation of the Moroberekan rice cultivar, which incorporates this resistance (Chen *et al.* 2000; Idowu *et al.* 2013).

### 1.3.3 Molecular mechanism of disease resistance

#### 1.3.3.1 Qualitative resistance

The first line of defence in a plant’s immune system is the ability to recognise “PAMPs” – pathogen associated molecular patterns. These are the chemical signatures of a broad range of pathogens, for example the chitin of fungal cell walls, or flagellin of bacterial flagellae (Boller and Felix 2009). The presence of compounds such as these in contact with pattern recognition receptors in the plant’s cell membranes ordinarily triggers an immune response such as callose deposition, to prevent the pathogen from establishing an infection (Jones and Dangl 2006; Niks *et al.* 2011). This first stage of defence prevents infection by the majority pathogenic microbe species, and forms

the basis of “non-host” resistance. Pathogen species which are adapted to infect the plant (i.e., pathogens for which the plant is a host species) are potentially able to shut down this PAMP triggered immunity using “effector” proteins, which typically contain an RXLR-EER motif (Jones and Dangl 2006). In the case of a susceptible host, the pathogen’s effector proteins will shut down PAMP triggered immunity and allow the pathogen to infect the host. However, if the host carries R-genes conferring resistance to the pathogen, the second line of defence in the plant immune system will take effect, operating within the cell cytoplasm (Jones and Dangl 2006). Pathogen specific R-genes encode NB-LRR proteins (proteins containing Nucleotide Binding and Leucine Rich Repeat domains), which interact with specific pathogen effectors and trigger a HR (Hammond-Kosack and Jones 1997). This second line of defence is known as effector triggered immunity. Unlike PAMP triggered immunity, which recognises highly conserved targets, effector triggered immunity is highly specific to particular effectors. The pathogen genes coding for these recognised effector proteins are termed “avirulence genes”. This nomenclature is convenient but misleading, as of course the genes evolved in order to allow the plant to overcome PAMP triggered immunity in susceptible hosts. The specificity of effector triggered immunity means that it is vulnerable to evasion via changes the amino acid sequence of pathogen effectors. Such changes may prevent the effector from performing its function of shutting down PAMP triggered immunity, but if it retains this functionality whilst evading recognition by the NB-LRR proteins encoded by the plant R-gene, then the pathogen is able to overcome the R-gene. Whisson *et al.* (2007) identified 425 DNA sequences encoding the RXLR-EER motif typical of effector proteins in the *P. infestans* genome. This large repertoire of effector genes may mean that functional redundancy allows for radiative evolution without compromising the function of existing effector genes. This may explain the rapid failure of the 11 potato “R Genes”, and the tomato genes *Ph-1*, and to some degree *Ph-2* (Foolad *et al.* 2008; Wastie 1991). It has often been supposed that if the “avirulence” gene recognised by a resistance gene were a molecule critical to the pathogen’s fitness, then any mutation would likely be deleterious to the pathogen, and that complex pathogen races able to overcome numerous resistance genes would therefore be less fit in the absence of selection pressure for this trait. There is some evidence that this may be the case in some instances, for example races of Tobacco Mosaic Virus capable of overcoming the *TM-2<sup>2</sup>* TMV resistance gene suffer serious fitness costs on susceptible plants (Johnson and Jellis 2013). However, this theory has not held true in the *P. infestans* – Potato pathosystem, where complex races able to overcome numerous potato R genes show no obvious lack of fitness (Fry 2008). This situation, combined with the rapid generation rate of *P. infestans*, facilitates it rapidly overcoming isolate-specific resistance genes (Fry 2008b; Goodwin *et al.* 1995).

### 1.3.3.2 Quantitative resistance

Whilst most qualitative R-genes operate by facilitation pathogen recognition and triggering the HR in the manner described above, quantitative resistance genes often function by up-regulating the synthesis of a wide range of defence compounds in the presence of a pathogen (Agrios 2004). A study of gene expression in potato following *P. infestans* inoculation found that a large number of genes associated with primary and secondary metabolism were up-regulated. Known functions included cell-wall modification and toxin synthesis (Tian *et al.* 2006). Whilst this study was not carried out on tomato, *S. lycopersicum* and *S. tuberosum* are closely related species, sharing 91.3% genetic sequence identity (Tomato Genome Consortium, 2012), so it is likely that many of the same processes take place in tomato. Several workers have reported a residual quantitative resistance to *P. infestans* conferred by defeated (qualitative) R-genes in potato (Rauscher *et al.* 2008; Stewart *et al.* 2003; Tan *et al.* 2008). Vleeshouwers *et al.* (2011) suggest three possible mechanisms to explain this phenomenon. They propose that the pathogen effector (avrulence gene) may have mutated such that it “partially avoids” detection by the host R-gene, although they do not elaborate on the precise mechanism of this. Alternatively, they propose that effector expression is reduced and/or delayed. Finally, they propose that the pathogen may have evolved a new gene in addition to the (unchanged) effector gene, which has the effect of modifying the host plant’s recognition response.

### 1.3.4 Other traits contributing to resistance

In addition to physiological or biochemical processes of disease resistance, an important contribution is made by physical characteristics of the plant. Brouwer *et al.* (2004) noted that different genotypes resulting from an *S. lycopersicum* x *S. habrochaites* cross had different ability to disperse moisture droplets on the leaves, and that this was strongly correlated with their blight resistance. Since *P. infestans* spores generally require liquid water for effective germination, and may be spread through a crop by rain splash, a hydrophobic leaf surface that prevents droplets from settling may help to reduce infection. Plant architecture can have a marked influence on blight susceptibility, with long internodes and an open morphology that facilitates drying of foliage helping to reduce vulnerability to blight and other diseases.

Grafting of tomato plants to resistant rootstocks is now widely used to provide protection from soil-borne diseases and abiotic stresses such as drought (Rivard *et al.* 2009). Additionally, grafted plants have shown increased resistance to some foliar diseases (Louws *et al.* 2010). Late-blight susceptible tomato scions grafted to a variety of rootstocks, including scion rootstocks, showed increased resistance to diseases including late blight, possibly due to increased production of secondary metabolites (Cao *et al.* 2010). Wild or specially bred rootstocks often increase nutrient

uptake and plant vigour, which may contribute to disease resistance in some cases (Guan *et al.* 2012) but the fact that autografts also exhibited enhanced resistance (despite reduced biomass production) suggests that this is not the mechanism. A more likely explanation is systemically increased expression in the scion of genes related to plant defence, possibly in response to the grafting “injury”, which has been documented in a range of species (Guan *et al.* 2012). However, grafting for late-blight resistance does not seem to have been widely tested to date.

## **1.4 Breeding for *P. infestans* resistance in tomato**

Three major resistance genes (*Ph-1*, *Ph-2*, and *Ph-3*) have been discovered and introgressed into horticultural cultivars (Foolad *et al.* 2008). Cultivated *S. lycopersicum* has a restricted genetic base which offers little scope for finding disease resistance genes (Ashrafi *et al.* 2009; Foolad 2010; Rick 1976), and these genes are all derived from the wild tomato species *S. pimpinellifolium*. All three genes show some degree of race-specificity (Kole 2007). Other proposed major resistance genes are discussed below.

### **1.4.1 *Ph-1***

*Ph-1* was derived from *S. pimpinellifolium* accessions “West Virginia 19” and “West Virginia 731” in the early 1950s following screening of a range of genetic material (Bonde and Murphy 1952; Gallegly and Marvel 1955). *Ph-1* is a dominant gene located at the distal end of Chromosome 7 (Peirce 1971). It offered complete resistance to T-0, the dominant *P. infestans* strain on tomato in the USA in the 1950s, and was first integrated into the cultivars “Rockingham”, “Nova” and “New Yorker”. However, evolution of new strains of *P. infestans*, against which *Ph-1* conferred no resistance, rendered the gene obsolete in resistance breeding in the United States and Europe (Walter and Conover 1952), and it is no longer considered useful (Foolad *et al.* 2014; Mutschler *et al.* 2006). The *Ph-1* gene does not appear to have been the subject of any recent studies, and the molecular mechanisms of its activity or inheritance do not appear to have been elucidated.

### **1.4.2 *Ph-2***

Soon after the discovery and defeat of *Ph-1*, resistance to *P. infestans* isolates which overcame *Ph-1* was discovered in *S. pimpinellifolium* accession “West Virginia 700” (Gallegly 1960). The resistance was initially believed to be multi-genic, owing to variability seen in phenotypes (Gallegly 1960), but was subsequently shown to be monogenic and dominant in inheritance studies by Turkensteen (1973), which also established that the resistance phenotype was variable at first, but became more clearly defined (and explicable in terms of a single, classically inherited gene) when plants reached eight weeks of age. Moreau *et al.* (1998) undertook a study to map *Ph-2* in which they demonstrated that the gene was partially dominant (F<sub>1</sub> progeny

showed susceptibility intermediate between that of the resistant and susceptible parents). The segregation ratios observed in the study provided further evidence of the monogenic nature of *Ph-2*, although owing to difficulties assigning intermediate phenotypes to “resistant” or “susceptible” classes, the authors did express caution with regard to this finding. The gene was subsequently mapped between CAPS markers dTG422 and dTG63, at the bottom of Chromosome 10 (Panthee & Foolad 2012). An F<sub>2</sub> mapping study carried out using a cross between “West Virginia 700” and a susceptible cultivar suggested that its resistance was in fact conferred by five QTLs, four of which were in the vicinity of the previously mapped *Ph-2* gene on Chromosome 10, but a fifth (accounting for 33.87% of phenotypic variation) was located near marker OPK14 on Chromosome 1.

Like *Ph-1*, *Ph-2* confers strong resistance against *P. infestans* race T-0, but additionally partial resistance against race T-1. The gene is incompletely dominant (Moreau *et al.* 1998). *Ph-2* confers partial, infection rate limiting resistance against several *P. infestans* strains (Laterrot 1975), and was found by Foolad *et al.* (2014) to limit the level of disease severity to 18.1 +/- 7.5% of the level on susceptible control plants in a range of different trials. Kole (2007) suggests that *Ph-2* resistance is most effective early in plant or disease development, becoming less effective later in the cropping period, although Turkensteen (1973) found that resistance of “West Virginia 700” seedlings to infection was approximately five times greater in eight week old than in six week old plants, but also that the variance was considerably greater in the older plant population. Expression of *Ph-2* resistance is complex and controlled by a number of additional factors (Laterrot 1975; Turkensteen 1973). Brusca (2003) reported that none of the *P. infestans* isolates held at the Mountain Horticultural Crops Research and Extension Center were able to completely overcome *Ph-2* (in contrast, several were able to overcome *Ph-3* and all overcame *Ph-1*).

Numerous cultivars carrying *Ph-2* have been developed, including “West-Virginia 63” (Gallegly 1964), “Caline” (Goodwin *et al.* 1995), “Legend”, “Centennial”, “Macline”, “Pieraline”, “Herline”, “Fline”, “Flora Dade”, “Heinz 1706”, “Campbell 28”, “Europeel” (Foolad *et al.* 2014), “Ferline”, “Fantasio”, “Losetto”, and “Lizzano” (Simon Crawford, Burpee Seeds Ltd., *pers. comm.*). Mutschler *et al.* (2006) state that owing to its limited effectiveness against many *P. infestans* strains, *Ph-2* is also becoming less favoured in resistance breeding. However, several workers have reported a stronger resistance when both *Ph-2* and *Ph-3* genes are present (Nowicki *et al.* 2013; Wagner 2012), and *Ph-2* remains important for this reason even if it is not of much value alone. The molecular mechanisms of the activity and inheritance of *Ph-2* do not appear to have been elucidated.

### 1.4.3 Major genes identified at AVRDC

Following a large, simple screening experiment at the Asian Vegetable Research and Development Centre (AVRDC) aimed at discovering new *P. infestans* resistance sources in tomato (AVRDC 1993), several resistant accessions were evaluated within a few years of each other. Putative genes designated as *Ph-3*, *4* and *5* were identified in *S. habrochaites* accession LA1033 and *S. pimpinellifolium* accession L3708. However, subsequent studies of these accessions found the resistances to be multigenic. Accordingly, the nomenclature of these genes changed several times between 1993 and 2005 (AVRDC 1993; AVRDC 1998; AVRDC 2003; AVRDC 2005) but it now appears that *S. pimpinellifolium* L3708 carries several resistance genes including *Ph-3*, which is now reasonably well characterised as a cluster of several genes, and *Ph-4*, which is less well characterised and does not appear to have been used in breeding (see below). The resistance of LA1033 was clearly shown to be distinct from that of L3708 by experiments in which LA1033 resisted infection by *P. infestans* isolates to which L3708 was susceptible (AVRDC 2005) but as this resistance had been shown to be multi-genic (Lough 2003) no individual genes in this accession have been designated as *Ph*- genes. The *Ph-3* and *Ph-4* resistance genes from L3708 are discussed below, as are two genes recently identified in *S. pimpinellifolium* accession PI 270443 by Merk *et al.* (2012), now designated as *Ph-5.1* and *5.2*.

### 1.4.4 *Ph-3*

The *Ph-3* resistance was named in 2002 (Chunwongse *et al.* 2002), and is conferred by a single partially dominant major gene (Chen *et al.* 2014). The resistance is derived from *S. pimpinellifolium* accession L3708, which was observed to exhibit strong resistance to a number of *P. infestans* strains during epiphytotics in Thailand (AVRDC 2005; Black *et al.* 1996a; Black *et al.* 1996b). Studies initially indicated that the resistance was conferred by a single gene (Black *et al.* 1996b). However, a mapping study by Frary *et al.* (Frary *et al.* 1998; Frary *et al.* 1998) found that resistance was a continuously varying trait in a cross between *S. pimpinellifolium* L3708 and the susceptible *S. lycopersicum* line NC23-2(93), and their results suggested that it was conferred by three QTLs located on Chromosome 6. However, the resistance has since been mapped to a single region on Chromosome 9, between CAPS markers TG328 and TG591 (Chen *et al.* 2014; Robbins *et al.* 2010). An additional strong resistance gene identified by Smilde *et al.* (2005) was later found near to *Ph-3* (discussed below) and is likely to be an important component of the resistance exhibited by *S. pimpinellifolium* L3708 (Kim and Mutschler 2006). (Foolad *et al.* 2014) reported that *Ph-3* reduced disease severity to 9.6 +/- 6.8% of that of susceptible plants in *S. pimpinellifolium* NC870 and to 14.1 +/- 4.8% of the control in *S. pimpinellifolium* L3708.



Owing to the strong resistance it confers against a wide range of *P. infestans* isolates, *Ph-3* is widely used in current blight-resistance breeding (Mutschler *et al.* 2006). The importance of zygosity appears to be dependent on *P. infestans* isolate, with heterozygous lines exhibiting complete resistance (like the homozygous parent) to US-11, but almost complete susceptibility to US-7 (Kim and Mutschler 2006). In Chen *et al.* (2014)'s recent mapping study, the resistance was found to be partially dominant. Other breeders have also reported anecdotally that resistance is stronger in the homozygous state (Wagner 2012).

Zhang *et al.* (2013) found that the resistance in L3708-derived line CLN2037B was located in a 74 kb region between markers Indel\_3 and P55. Marker TG591 is within this region, whereas marker TG328 is approximately 40 kb outside it, indicating that where the TG328-derived marker genotype differs from the TG591-derived marker genotype, the latter is slightly more likely to correctly indicate *Ph-3* genotype. However, the distance between the two markers is small (approximately 60 kb, 0.4 cM) so recombination events between them would be expected to be uncommon. Four candidate resistance genes were identified within this interval with homology to the *Rpi-vnt1.1* *P. infestans* resistance gene in potato, and the *Tm-2<sup>2</sup>* tomato mosaic virus resistance gene in tomato. Virus-induced gene silencing confirmed that one or more of these genes was likely to be responsible for *P. infestans* resistance in CLN2037B, as in two separate experiments, 21 of 29, and 24 of 31 CLN2037B plants infected with a *Tm-2<sup>2</sup>*-family gene-silencing vector showed susceptibility when inoculated with *P. infestans*. A further study by Zhang *et al.* confirmed that *Ph-3* was indeed one of the four candidates identified between Indel\_3 and P55, namely ORF3 (Zhang *et al.* 2014). This study also demonstrated that the *Ph-3*/ORF3 gene carried by the *S. pimpinellifolium* line L3708 (which is known to carry *Ph-3*) showed 85-95% nucleotide identity to four homologous resistance gene analogues in the susceptible line Heinz1706. This indicates that susceptible tomato lines are likely to carry non-functional allelic variants of the *Ph-3* gene (as opposed to lacking the gene altogether, or carrying it but lacking factors necessary for its expression).

#### **1.4.5 *Ph-4***

Mapping work by Kole *et al.* (2006) confirmed that *S. pimpinellifolium* accession L3708 does indeed carry a qualitative *P. infestans* resistance gene in addition to *Ph-3*, which was mapped to Chromosome 2 and named *Ph-4*. Additional factors controlling the expression were also identified. The gene does not appear to have been used in breeding programmes to date.

#### **1.4.6 *Ph-5.1* and *Ph-5.2***

Two resistance genes have been identified in *S. pimpinellifolium* accession PI 270443 (which is also referred to as "PSLP153" in some) and have been shown to confer strong *P. infestans*

resistance, equivalent to the resistance from *Ph-2* and *Ph-3* combined (Merk *et al.* 2012). In a range of experiments, PI 270443 suffered 6.7 (+/- 7.0) % disease severity compared to approximately 100% defoliation in susceptible controls. The resistance is highly heritable ( $H^2 = 0.86$ ) and the genes appear to be partially dominant as the mean susceptibility of the  $F_1$  progeny from a cross to a susceptible line was closer to the resistant than susceptible parent, whereas the mean susceptibility of the  $F_2$  population was closer to the susceptible (Merk and Foolad 2012). The genes have been mapped to Chromosomes 1 and 10. The gene on Chromosome 1 was mapped to a 23 cM section between markers SSRW11 at position 106.1 to cTOE7J7 at position 129.1, and the gene on Ch. 10 to a 38 cM region between TMA0040 at position 106.1 to SSR223 at position 67.1 (Merk *et al.* 2012). The gene on Chromosome 10 was located in the same chromosome region as *Ph-2*, and further fine-mapping work is needed to verify that it is another distinct gene (Merk *et al.* 2012). The gene on Chromosome 1 has been provisionally named *Ph-5.1* and the gene on Chromosome 10 has been provisionally name *Ph-5.2* (Nowicki *et al.* 2012). Efforts are currently underway to develop breeding lines and cultivars using the new resistance genes (Nowicki *et al.* 2013).

#### 1.4.7 *P. infestans* resistance QTLs in tomato

**Table 1.2** **Error! Reference source not found.** describes studies which have been carried out to screen tomato germplasm, and in some cases, to map any resistance it carries to particular chromosomal locations. Most of the QTLs described below are not as strong as the *Ph-* genes and alone cannot prevent a *P. infestans* infection from occurring and spreading. However, they can slow the rate of spread of infection should it occur, and where several minor genes are “stacked” or “pyramided” in a tomato cultivar, reduce the occurrence of the disease to a point at which it is no longer a problem. Unfortunately, minor genes have found little use in tomato breeding to date, largely because those identified have often been in undesirable “wild” backgrounds, and linkage drag of maladaptive traits such as late fruit maturity and over-vigorous vegetative growth have been a problem (Brouwer and St Clair 2004). Johnson *et al.* (2012) note that QTLs causing multiple effects may be due to pleiotropy, which may render the QTL useless for breeding if the resistance is linked to an undesirable trait, or may be due to close linkage or, in the case of non-inbred individuals (especially tetraploid potato clones (Oberhagemann *et al.* 1999)) multiple co-dominant alleles at the same locus, each with different phenotypic effects.

**Table 1.2: Previously identified *P. infestans* resistance genes and QTLs from tomato**

<b>Study</b>	<b>Accession</b>	<b>Findings</b>	<b>QTL Location</b>
Johnson <i>et al.</i> (2014)	Wapsinicon Peach Matt's Wild Cherry ( <i>S. lycopersicum</i> var. <i>cerasiforme</i> ) Pruden's Purple (unknown origin. Large fruit).	Substantially reduced lesion expansion rate in DL tests compared with susceptible control. Similar to Mountain Magic. Wapsinicon Peach is probably White Peach, which is probably an <i>S. lycopersicum</i> x <i>S. pimpinellifolium</i> cross.	Unknown
Bonde and Murphy (1952); Gallegly and Marvel (1955); Peirce (1971)	<i>Pimpinellifolium</i> "West Virginia 19" and "West Virginia 731"	Discovery of <i>Ph-1</i> , conferring dominant resistance to <i>P. infestans</i> race T0 only.	Distal end (long arm) of Ch. 7. Mapped using morphological markers by Peirce (1971). Mapping was not very precise.
Gallegly and Marvel (1955)	<i>Pimpinellifolium</i> "West Virginia 700"	<i>Ph-2</i> . Reduces the rate of disease development (Goodwin <i>et al.</i> 1995; Black <i>et al.</i> 1996).	<i>Ph-2</i> gene, on Ch. 10 (Moreau <i>et al.</i> 1998) between markers CP105 and TG233 (roughly 61-69 cM)
Brouwer <i>et al.</i> (2004)	Selection MD1 from <i>S. habrochaites</i> LA2099	Conducted DL tests in which the assessed lesion length. Conducted whole plant tests which assayed foliage and stem DS on plants in growth cabinets.	Numerous QTLs conferring Late Blight resistance were identified: C1: 0-52; 53-67; 80-83; 112-145 cM C2: 10-54; 69-74 cM C3: 9-49; 57-94 cM C4: 0-52 cM C5: 31-80 cM C6: 0-19 (from S parent); 19-72 cM C7: 55-67 cM C8: 3-47; 63-84 cM C9: 32-54; 55-81 cM C10: 2-29 cM C11: 27-77 cM C12: 7-9; 33-57 cM

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**Table 1.2 (Continued)**

<b>Study</b>	<b>Accession</b>	<b>Findings</b>	<b>QTL Location</b>
Brouwer and St Clair (2004)	Selection MD1 from <i>S. habrochaites</i> LA2099	<i>Lb4</i> gave resistance to leaf and stem blight. Mapped to same location as <i>Pi1</i> in potato (Leonards-Schippers <i>et al.</i> 1994). Nearby QTLs confer more spreading habit, less dense canopy, earlier maturity reduced yield and fruit size.	<i>lb4</i> between TG182 and CT194 (6.9 cM) centred on TG609. This is around the middle third of C4 (ranges from 32.9 to 85.5 on SGN).
	Selection MD1 from <i>S. habrochaites</i> LA2099	Created three sub-NIL families to fine-map <i>lb4</i> , <i>lb5b</i> , and <i>lb11b</i> . <i>lb5b</i> was the most consistently identified QTL. Leaves only (not stems). Nearby QTLs confer earlier flowering, reduced yield and fruit size (possibly just one gene, or several tightly linked).	<i>lb5b</i> between TG69a and TG413 (8.8 cM) centred between TG23 and CT80 (1.4 cM). This is the bottom half of C5. Ranges from 63.92 to 128.45 on SGN.
	Selection MD1 from <i>S. habrochaites</i> LA2099	Additional less well supported QTLs.	Possible extra QTLs between CT80 and TG185 (8.4 cM) on Ch. 5, (bottom end of C5 – 75.0-119.0 on SGN). Also near TG393 on Ch. 11 (bottom of C11 – 68.8-107.0
	Selection MD1 from <i>S. habrochaites</i> LA2099	<i>Lb11b</i> confers resistance to stem and (mainly) foliage blight. Nearby QTLs for more open canopy and reduced fruit weight.	<i>lb11b</i> between TG194 and TG400 (15.1 cM) probably between CT182 and TG147 (5.2 cM). Second quarter of C11 – between 27.0 and 55.2 on SGN.
Lobo and Navarro (1986)	<i>S. habrochaites</i> PI 251305	Significantly lower infection rate compared with <i>S. lycopersicum</i> “Licato” susceptible control.	Unknown
Lobo and Navarro (1987)	<i>S. habrochaites</i> LA1252, LA1253, LA1255, LA1223, LA1265, LA1254, LA1624, LA1625, LA2092, LA2098, PI126445.	Line exhibited low infection frequency. F <sub>1</sub> progeny from a cross with more susceptible <i>S. lycopersicum</i> produced intermediate progeny. Replication not stated, and no measure of significance. Not incorporated into any commercial cvs.	Unknown

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**Table 1.2 (Continued)**

<b>Study</b>	<b>Accession</b>	<b>Findings</b>	<b>QTL Location</b>
Abreu <i>et al.</i> (2008)	<i>S. habrochaites</i> accession BGH6902 crossed to <i>S. lycopersicum</i> F2.	Conducted crosses to susceptible <i>S. lycopersicum</i> . Classical genetics suggested the involvement of 28 genes in blight resistance. Heritability ( $R^2$ ) was 9.06 %.	Unknown.
Elsayed <i>et al.</i> (2011)	Five inbred from a cross of <i>S. lycopersicum</i> × <i>S. habrochaites</i> f. <i>glabratum</i> accession BGH6902.	Related to Abreu <i>et al.</i> (2008). Evaluated the combining ability of five RILs derived from Abreu's F <sub>2</sub> population with five parent lines including NC1- and NC2-CELBR. Results suggest that QTL resistance from <i>S. habrochaites</i> is recessive; AUDPC of the five RILs was 100-200, and of S parents was 700-800 % Days and AUDPC of most F <sub>1</sub> s had AUDPCs close to the susceptible parent. NC parents were 25-50, and the AUDPCs of crosses with them were closer to those of the RILs.	Unknown.
Li <i>et al.</i> (2011)	<i>S. habrochaites</i> LA1777	Screened LA1777 × <i>S. lycopersicum</i> introgression lines. Measured lesion size and infection efficiency in DL expts. Very few lines significantly differed from the susceptible parent with regard to IE. <i>Rlbq4b</i> is probably new, but the other four QTLs co-localise with QTLs identified by Brouwer <i>et al.</i> (2004).	<i>Rlbq4a</i> - Top of Ch. 4 <i>Rlbq4b</i> - Bottom of Ch. 4 <i>Rlbq7</i> - Top of Ch. 7 <i>Rlbq8</i> - Bottom of Ch. 8 <i>Rlbq12</i> - Middle of Ch. 12
	<i>S. habrochaites</i> LA2099, LA1033	Both significantly more resistant than control when exposed to <i>P. infestans</i> T <sub>1,2,4</sub> , but not used further as study focussed on LA1777. LA1033 was almost completely susceptible to the most virulent race (T <sub>1,2,3,4</sub> ). LA1033 may carry a <i>Ph-3</i> allele.	Unknown.
	<i>S. lycopersicoides</i> LA2951	The authors mention that they have found that LA2951 has <i>P. infestans</i> resistance, but do not give data.	Unknown.

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**Table 1.2 (Continued)**

<b>Study</b>	<b>Accession</b>	<b>Findings</b>	<b>QTL Location</b>
Smart <i>et al.</i> (2007)	<i>S. pennellii</i> LA716	More resistant genotypes had fewer lesions which expanded more slowly and have limited sporulation. Accounted for 25% of variance in F2 mapping pop (disease severity at 14 dpi = 15 to 90%) and an IL carrying T1556 had disease severity of 75% compared to 90% (control and many other ILs) at 14 dpi under very high disease pressure, and 35% compared to 90% under moderate disease pressure. Not very strong resistance.	Middle of Ch. 6, near marker T1556. According to SGN, this is between 40.36 and 63.95.
Merk and Foolad (2012); Merk <i>et al.</i> (2012)	<i>S. pimpinellifolium</i> PI 270443	Highly heritable ( $H^2 = 0.86$ ). PI 270443 suffered 6.7 (+/- 7.0) % defoliation compared to approx. 100% in susceptible controls. Resistance similar to that of ( <i>Ph-2</i> , <i>Ph-3</i> ) control. Resistance is probably partially dominant (as F <sub>1</sub> was closer to resistant than susceptible parent, but F <sub>2</sub> mean was closer to susceptible). The QTL on Ch. 10 may be <i>Ph-2</i> or an allele thereof (it's in approximately the right place and PI 270443 has the <i>Ph-2</i> markers).	Ch. 1, in a 23 cM section between SSRW11 at position 106.1 to cTOE7J7 at position 129.1 (lower-mid region), and on Ch. 10 between TMA0040 at position 106.1 to SSR223 at position 67.1 (38 cM).
Foolad <i>et al.</i> (2014)	<i>S. pimpinellifolium</i> PI 270441, PI 270445, PI 270446, PI 270447, PI 270448, PI 270449, PI 270451.	<1% foliage blight in severely infected field trials in two years. All carry <i>Ph-2</i> but not <i>Ph-3</i> . Far higher resistance than <i>Ph-2</i> only control.	<i>Ph-2</i> plus presumed unknown additional gene(s).
	<i>S. pimpinellifolium</i> LA2533	<1% foliage blight in severely infected field trials in two years. Carries <i>Ph-3</i> , but far higher resistance than <i>Ph-3</i> only control.	<i>Ph-3</i> plus presumed unknown additional gene(s).
	<i>S. pimpinellifolium</i> PI 163245, PI 224710 <i>S. Pimpinellifolium</i> PI 270450, PI 270443, PI 270439, PI 270442	High blight resistance in most trials. Does not carry <i>Ph-2</i> or <i>Ph-3</i> . 4.2 – 12.1% blight infection over multiple trials. Carry <i>Ph-2</i> but not <i>Ph-3</i> . Performed better than <i>Ph-2</i> only control (18.1%)	Not <i>Ph-2</i> or <i>Ph-3</i>  <i>Ph-2</i> plus presumed unknown additional gene(s).

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**Table 1.2 (Continued)**

<b>Study</b>	<b>Accession</b>	<b>Findings</b>	<b>QTL Location</b>
Chen <i>et al.</i> (2008)	<i>S. pimpinellifolium</i> L3708	Carries Putative <i>Ph-4</i> gene as well as <i>Ph-3</i> , as it was resistant to isolates that were virulent against a <i>Ph-3</i> carrying line developed from L3708. The <i>Ph-3</i> line seemed unexpectedly susceptible, however.	Location unknown. Gene provisionally named <i>Ph-4</i> , but may be <i>Ph-3</i> .
	<i>S. pimpinellifolium</i> LA1033	Used as a differential, this accession exhibited resistance to a number of <i>P. infestans</i> isolates that were avirulent on differentials carrying <i>Ph-1</i> , <i>Ph-2</i> , <i>Ph-3</i> and another putative resistance gene (provisionally <i>Ph-4</i> ).	Location unknown. Gene provisionally named <i>Ph-5</i> .
Kim and Mutschler (2000)	<i>S. habrochaites</i> LA1033-2	Moderate to strong resistance to multiple <i>P. infestans</i> isolates.	Unknown
	<i>S. pimpinellifolium</i> L3708	Strong resistance to multiple <i>P. infestans</i> isolates.	Unknown
Oyarzun <i>et al.</i> (1998)	<i>S. lycopersicum</i> cv. Peralbo	Exhibited resistance (no sporulating lesions >1 cm diameter after 1 week) to a few isolates that infected a susceptible control.	Unknown
Johnson <i>et al.</i> (2012)	Selection MD1 from <i>S. habrochaites</i> LA2099	Identified 17 groups of co-localised QTLs within the previously identified <i>lb5b</i> and <i>lb11b</i> QTLs (Brouwer and St Clair, 2004). Identified separate QTLs for stem and foliage resistance. 4 of 17 groups actually came from S parent. 3 of the groups (from <i>lb11b</i> ) were stable expressed across most experimental conditions.	All within the windows identified in Brouwer and St Clair 2004.

#### 1.4.8 Use of *P. infestans* resistance QTLs in tomato

Despite the fact that many of the *P. infestans* resistance QTLs discussed in **Table 1.2** show the potential to provide useful levels of resistance, none of them appear to have been used in breeding programmes. Nowicki *et al.* (2013) also assert that no breeding programmes they were aware of had made use of *P. infestans* resistance QTLs. Part of the reason for this slow uptake is that QTL-mediated disease resistance tends to have low heritability and is inherently difficult to transfer from wild relative to cultivated crop background using conventional phenotypic selection. This is because of the marked influence of environmental variation (including the pathogen genotype) and genotype by environment interaction on the disease resistance phenotype. For QTLs with small individual effects to produce a useful level of disease resistance in a plant, it is necessary for a cultivar to carry several such QTLs. If lines in a breeding programme are selected based on their phenotype only, and the QTL genotype is not known, then it is difficult to select appropriate lines to combine (or “pyramid”) multiple QTLs to give useful levels of resistance (Niks *et al.* 2011). In contrast, the phenotypic expression of major genes for disease resistance is often obvious in field- or greenhouse-grown plants, making phenotypic selection relatively easy. For this reason, most early potato and tomato breeding programmes aimed at *P. infestans* resistance made use of the R or *Ph*- genes only (Fry 2008b; Nowicki *et al.* 2013), although the rapid defeat of single R genes by new *P. infestans* strains prompted an interest in QTL-mediated partial resistance in potato breeding (Leonards-Schippers *et al.* 1994; Turkensteen 1993).

### 1.5 Project Aims

As noted above, there is a lack of knowledge about the *P. infestans* population on tomato in Britain, and whether host specialisation on tomato versus potato occurs in Britain. There is also a lack of good quality blight-resistant tomato cultivars adapted for, and available to, British amateur tomato growers. This project was a collaboration between Bangor University, the Sarvari Research Trust ([www.sarvari-trust.org](http://www.sarvari-trust.org)), Burpee Europe Ltd. ([www.burpee.com](http://www.burpee.com)) and Pro-Veg Seeds Ltd. ([www.provegseeds.com](http://www.provegseeds.com)) and aimed to address both of these challenges.

The following chapters are as follows:

- **Chapter 2** describes field trials and molecular markers screening conducted over four years to identify tomato germplasm and breeding lines with *P. infestans* resistance and work with Pro-Veg and Burpee to develop novel blight-resistant tomato varieties for the British climate.
- **Chapter 3** describes a three year long citizen-science project in which members of the public sent samples of *P. infestans* infected tomato a potato to the authors for genotyping



in order to characterise the *P. infestans* population on tomato in Britain, and examine whether it differed from that on potato.

- **Chapter 4** describes detached-leaflet inoculation experiments carried out to investigate the ability of tomato cultivars with different combinations of *P. infestans* resistance genes to resist infection by several different *P. infestans* genotypes collected during this project.
- **Chapter 5** describes a QTL mapping study undertaken to investigate the genetic basis for the *P. infestans* resistance seen in Koralik, an “heirloom” tomato cultivar.

## 2 Field Assessment and Genotyping of a range of tomato germplasm

### 2.1 Introduction

As discussed in **Chapter 1**, tomatoes are one of the most popular crops with Western amateur gardeners (Staub 2010). Tomato cultivars without resistance genes are highly susceptible to late blight disease, caused by *P. infestans*. Plants grown in greenhouses are at substantially lower risk of infection than those grown outdoors, as *P. infestans* spores require liquid water on foliage, or 100% humidity, to infect plants, and the pathogen grows and reproduces fastest at temperatures below 25 °C (Fry 2008b). Greenhouse conditions will normally therefore be too warm and dry for *P. infestans* to grow effectively (Collins 2013; Nelson 2008). However, amateur gardeners frequently do not have access to a glasshouse in which to grow tomatoes. Furthermore, no effective synthetic fungicides capable of effectively protecting an outdoor crop are available to amateur gardeners in Britain (many of whom do not wish to use chemical protection in any case). Copper-based sprays are available to amateurs, but they have significant environmental and human toxicity and are not recommended (Pears 2005; RHS 2014). Therefore, tomato cultivars with genetic resistance to *P. infestans* may be the most appropriate option for many amateur growers, and in any case, genetically resistant cultivars can usefully be grown as part of an integrated approach to late blight management which may also include the other control options discussed in **Chapter 1** as well.

A number of major genes and QTLs conferring resistance to *P. infestans* have been discovered, and are discussed in **Chapters 1** and **5**. The major-genes *Ph-1*, *Ph-2*, and *Ph-3* have all found extensive use in tomato breeding programmes (Nowicki *et al.* 2013). Owing to the fact that most currently extant *P. infestans* races are capable of completely overcoming *Ph-1*, it is now considered defeated and no longer used in tomato breeding (Foolad *et al.* 2008; Nowicki *et al.* 2013; Nowicki *et al.* 2012). However, *Ph-2* and/or *Ph-3* are present in most resistant cultivars grown today. *Ph-3* confers stronger resistance to most *P. infestans* races than does *Ph-2*, which tends to slow the spread of an infection rather than prevent it (Foolad *et al.* 2014; Moreau *et al.* 1998). A number of workers have noted that the strongest resistance is obtained from a combination of both *Ph-2* and *Ph-3* (Brusca 2003; Foolad *et al.* 2014; Nowicki *et al.* 2013; Wagner 2012).

Zygosity of *Ph-2* and *Ph-3* appears to influence the strength of the resistance conferred by both genes. Moreau *et al.* (1998) found that F<sub>1</sub> hybrids heterozygous for *Ph-2* showed disease symptoms intermediate between the homozygous resistant and susceptible parents, indicating

that *Ph-2* is partially dominant, although Turkensteen (1973) found that the gene acted in a dominant fashion. Kim and Mutschler (2006) found that the importance of *Ph-3* zygosity depended on the *P. infestans* isolate, with the gene conferring completely dominant resistance to *P. infestans* US-11, but completely recessive resistance to US-7. Several other *P. infestans* strains produced intermediate responses. Brusca (2003) examined the *P. infestans* resistance present in “Richter’s Wild Tomato” (which is likely to have originated from an *S. lycopersicum* x *S. pimpinellifolium* cross) and showed that the resistance was allelic to the *Ph-2* gene in cv. West Virginia 63. The study found that the foliage disease severity on recombinant progeny lines containing the gene in the homozygous and heterozygous state were equivalent in two field tests, but that detached leaflet tests indicated incomplete dominance.

At the outset of this project, the range of blight-resistant tomato cultivars available to gardeners in Britain was very limited, and those that were available (for example, Ferline and Losetto) had only weak to moderate, monogenic resistance, and would frequently fail to give adequate performance under conditions conducive to *P. infestans* growth and spread (Simon Crawford, Burpee Europe Ltd., Foston-on-the-wolds, *pers. comm.*; David Shaw, Sarvari Research Trust, Bangor, *pers. comm.*). Therefore, a major objective of this project was the development of novel tomato cultivars with strong, multi-gene blight-resistance. Burpee Europe Ltd, Foston-on-the-Wolds, UK (henceforth “Burpee”) and the Sarvari Research Trust, Bangor, UK acted as industry partners in this breeding project. Much of the tomato variety development was carried out by Simon Crawford of Burpee and of Pro-Veg Seeds Ltd., Sawston, UK (henceforth “Pro-Veg”), with additional creative and financial input by John Burrows and Barrie Smith of Pro-Veg. In the interests of producing cultivars fit for market within the timescale of the project, many of the germplasm lines tested were existing cultivars, with resistance likely to be due to one or both of the two widely used resistance genes *Ph-2* and *Ph-3*. Wild-tomato derived lines suspected to contain other resistance genes were tested to evaluate their potential for use in future breeding work, and a mapping study was performed on one such line, “Koralik”, in an attempt to learn more about the resistance genes/QTLs present in this cultivar (discussed in **Chapter 5**). To complement and inform the breeding work by Simon Crawford, field assessment and genotyping of germplasm and breeding material was carried out by James Stroud at Bangor University as part of this research project, and is described here.

The broad aim of this project was to develop novel tomato cultivars with strong, durable blight-resistance and desirable horticultural characteristics (primarily fruit flavour, colour, shape, and earliness, and also reasonable yield). The aims of this series of trials and genotyping work carried out at Bangor University and discussed here were as follows:

- a. To identify germplasm with high levels of *P. infestans* resistance in the field.
- b. To test germplasm for the *Ph-2* and *Ph-3* genotype using molecular markers.
- c. To test new lines bred by Burpee and Pro-Veg for field resistance and *Ph-2* and *Ph-3* genotype.

## **2.2 Materials and Methods**

### **2.2.1 Overview of tomato field trials**

A limited field and polytunnel trial of germplasm was carried out in 2011, followed by larger trials of germplasm and breeding lines in 2012 and 2013, and a final field trial of lines developed during the project in 2014 (**Table 2.1**). The field trial in 2011 was conducted prior to commencement of the PhD project. The trial design was conceived by Simon Crawford, John Burrows and David Shaw in early 2011, with additional input from James Stroud. The trial setup and all data collection was carried out by James Stroud later in 2011. Trials in 2012, 2013 and 2014 were largely designed and conducted by James Stroud. The 2014 trial consisted mainly of material developed by Burpee during the course of this project and under consideration for registration and commercial release from 2015 onwards. This trial was conducted primarily as a commercial demonstration of new varieties, and as such its design was not optimised for statistical rigour.

**Table 2.1: Summary of the trials conducted in each year.**

*No. of Gtpes.* indicates the number of tomato lines included in the experiment, including susceptible controls. *No. of Reps* indicates the intended number of replicate plants or groups of plants for each line. In some instances the number of plants deviated from this number slightly.

Year	Trial Setting	No. of Gtpes.	No. of Reps	Sowing and Planting Date	Transplant Age	Inoculation Date	No of Assess.	First Assessment	Last Assessment
2011	Field	59	1 x (3) <sup>a</sup>	S: 15 <sup>th</sup> April P: Early June	8 Weeks	(20 <sup>th</sup> Aug) <sup>c</sup>	4	28 <sup>th</sup> Aug.	21 <sup>st</sup> Oct.
2011	Polytunnel	57	1 x (3) <sup>a</sup>	S: 15 <sup>th</sup> April P: 10 <sup>th</sup> Aug.	17 Weeks	N/A <sup>d</sup>	5	14 <sup>th</sup> Aug.	11 <sup>th</sup> Sept.
2012	Field	40	8	S: 16 <sup>th</sup> April P: 29 <sup>th</sup> June – 1 <sup>st</sup> July	10 Weeks	22 <sup>nd</sup> Sept.	5	29 <sup>th</sup> Sept.	4 <sup>th</sup> Nov.
2013	Field	67	8	S: 17 <sup>th</sup> April P: 24 <sup>th</sup> - 26 <sup>th</sup> June	9 Weeks	24 <sup>th</sup> Sept.	3	28 <sup>th</sup> Sept	21 <sup>st</sup> Oct.
2014	Field	28	2 x (3) <sup>a</sup> 3 x (72) <sup>b</sup> 1 x (2) <sup>b</sup>	S: 15 <sup>th</sup> April P: 25 <sup>th</sup> – 26 <sup>th</sup> June	9 Weeks	25 <sup>th</sup> Aug.	2	9 <sup>th</sup> Oct.	28 <sup>th</sup> Oct.

<sup>a</sup> Numbers in brackets indicate number of pseudoreplicate plants within each group. <sup>b</sup> Numbers in brackets indicate number of individual (unreplicated) F<sub>2</sub> plants in family. <sup>c</sup> The 2011 field trial was spray inoculated on the 20<sup>th</sup> of August, although some plants had already become infected by natural spread from a neighbouring experiment. <sup>d</sup> The polytunnel trials became infected by natural spread from neighbouring infected potato plants soon after planting.

## 2.2.2 Selection and propagation of germplasm

### 2.2.2.1 Selection of 2011 germplasm

Tomato germplasm was selected prior to this study and propagated by Simon Crawford and John Burrows of Pro-Veg. It consisted of 42 of Pro-Veg's breeding lines (from 4 parental combinations), nine other breeding lines from Pro-Veg, and eight named cultivars and tomato rootstocks from other sources, totalling 59 lines in all. With a few exceptions (see raw data in **Supplementary materials**), six plants of each line were raised or received from Pro-Veg. Of these, three were planted outdoors to mimic typical horticultural conditions, and three were grown in a polytunnel where heavy infection pressure could be guaranteed. For some cultivars, fewer than six plants were received from Pro-Veg or successfully germinated from seed, and priority was given to ensuring that three plants were present in the field. Plants were received as six week old seedlings in 10 cm pots, and were planted out in their final positions immediately in the case of the polytunnel, and within two weeks in the case of the field trial.

### 2.2.2.2 Selection of 2012 germplasm

Searches of published literature and online grey literature were carried out to identify germplasm for inclusion in field trials. Material was considered for inclusion if it met one or more of the following criteria:

- a. Material reported to carry *P. infestans* resistance genes or QTLs.
- b. Material reported in primary literature to have shown resistance to *P. infestans* in previous field assessments.
- c. Material reported to have resistance to *P. infestans* in grey literature (descriptions from seed suppliers, online discussion forums, magazines, and web articles).

Material which was identified by James Stroud for consideration is given in **Supplementary materials**. Material to be grown for use in field trials was selected from this list and propagated, subject to seed availability, by Pro-Veg, along with some additional material identified by Simon Crawford and John Burrows of Pro-Veg. The final germplasm list for the 2012 field trial included 38 tomato varieties selected according to the above criteria, plus two (Ailsa Craig and Red Alert) with no documented blight resistance which were included as susceptible controls. In most cases, eight replicate plants of each line were used, although in some cases (see raw data in **Supplementary materials**) fewer plants were available or successfully established in the field.

### 2.2.2.3 Selection of 2013 germplasm

Germplasm for the 2013 trial was jointly selected by James Stroud and Simon Crawford. Trial material consisted of 69 tomato lines, mostly commercially available *S. lycopersicum* cultivars, but also including unreleased breeding material developed by Pro-Veg. and Burpee, and several *S. pimpinellifolium* lines (see germplasm list in **Supplementary materials**). Germplasm was selected on the basis of reported blight tolerance, although in most cases the genetic basis of this was not known. “Ailsa Craig” and “Red Alert” were included as susceptible controls.

### 2.2.2.4 Selection of 2014 germplasm

The 2014 field trial served primarily as a final evaluation and commercial demonstration of lines developed by Burpee between 2011 and 2014, as well as some additional commercial cultivars for comparison, and breeding lines of interest to Burpee Europe Ltd. (see raw data in **Supplementary materials**)

## 2.2.3 Field and Polytunnel Trial Designs

### 2.2.3.1 Location and soil treatment

All trials were carried out at Henfaes Research Centre, Bangor University, Abergwyngregyn, Gwynedd (henceforth “Henfaes”). Henfaes is located on the North Wales coast at 53° 14'N, 4° 01'W, and has a mild maritime climate with 1250 mm mean annual rainfall (Millett *et al.* 2012).

Two trial plots were used in different years. The 2011 field trial took place in a large open field to the east of the site, known as “Beudy Mawr”, and the trials in 2012-2014 took place in a smaller, more sheltered field to the west of the site, known as “Harper’s Field” (**Figure 2.1**). Trial design and management in each year is described below. The 2012, 2013 and 2014 trials were conducted on a rectangular stony loam site measuring 14 m x 50 m. The site was long-term pasture prior to ploughing for the experiment in 2012 and it was cultivated using a tractor and power harrow between experiments. The trial site was covered with weed suppressant membrane, which was left in place year-round between trials to prevent turf re-establishment, being removed briefly to allow for cultivation immediately prior to planting in 2013 and 2014.

At both sites, the land was not fertilised or irrigated during the trials, although plants were given fortnightly 100 ml doses of Miracle-Gro liquid feed (Scotts Miracle-Gro, Marysville, Ohio, USA) at 4x the recommended concentration during the first four weeks of establishment, and in 2014 were watered by hand as required whilst establishing.



**Figure 2.1: The location of trial plots at Henfaes Research Centre**

a. 2011 Polytunnel Trial, b. 2012, 2013 and 2014 field trials in “Harper’s Field”, c. 2011 field trial in “Beudy Mawr”. Plots not drawn to scale. Aerial photo from [www.google.co.uk/maps](http://www.google.co.uk/maps).

### 2.2.3.2 2011 Field trial design and protocol

Plants were grown outdoors on a stony loam which had grown potatoes in the previous year. The soil was reasonably moist, free of weeds, and in adequate nutrient status, so no preparation was undertaken beyond removing larger volunteer potato plants. The trial plot (**Figure 2.2a**) measured 5 by 15 m and was covered with weed-suppressant membrane. Tomato plants were planted through holes cut in the membrane, spaced at 0.5 m between each of the three plants in a group, 0.75 m between groups. The groups were arranged in the field arbitrarily. Plants were planted out in the field in early June. Plants were watered when they were first planted, but after planting they were rainfed with no irrigation. The area was kept free of large weeds. Initial infection took place without intervention, from adjacent potato trials which were inoculated with a 13\_A2 *P. infestans* strain in early August and soon became heavily infected. However, soon after initial infection, it became apparent that inoculum pressure was likely to be greater at the end of the tomato trial nearest the potato trial, so to ensure a more even pressure, the entire tomato trial was inoculated with a 13\_A2 spore suspension (concentration not determined) prepared from infected potato foliage from the adjacent experiment, and applied with a watering can and fine rose on the 20<sup>th</sup> of August.

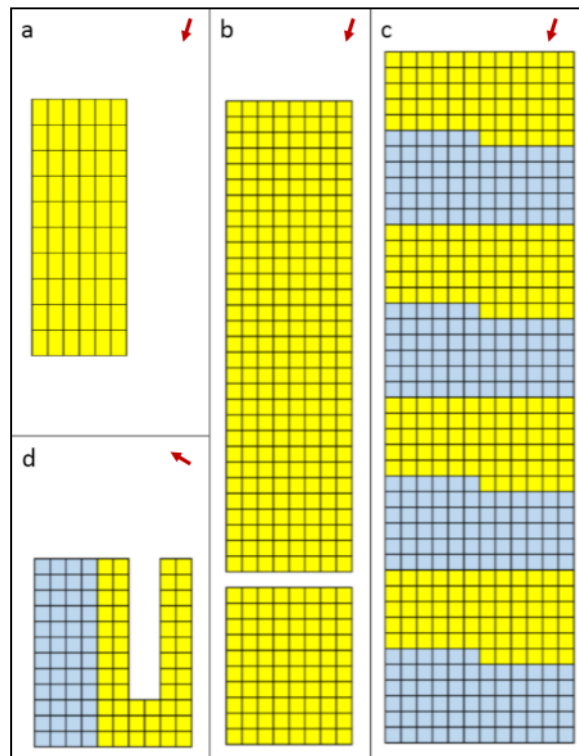


### 2.2.3.3 2011 Polyunnel trial design and protocol

The polyunnel trial was carried out in a 5 m x 20 m polythene tunnel. The conditions in the tunnel were managed to ensure a controlled, severe epiphytotic by maintaining a high humidity level by keeping ventilation to the minimum commensurate with temperature regulation, and by frequent overhead misting of the plants using an automated sprinkler system, which also served for irrigation. Potato plants heavily infected with a 13\_A2 *P. infestans* strain were kept in the tunnel from a previous experiment. Patches of these were left to provide an inoculum source. After receipt from Pro-Veg, the experimental plants were transferred into 7 litre pots in a general-purpose peat-based compost. Plants were fed with “Miracle-Gro” soluble plant food (Scotts Miracle-Gro, Marysville, Ohio, USA) approximately weekly, diluted as directed by the manufacturer. The three plants of each line were placed (in pots) in a close group beneath a sprinkler head on the 18<sup>th</sup> of August, when they were approximately 18 weeks old. Plants were supported with bamboo canes as required, but not pruned. The groups of three were arranged arbitrarily in the polyunnel, and groups of plants from the ends of the tunnel were swapped with groups in the centre at approximately weekly intervals to minimise the “edge effect” of lower humidity and lower inoculum pressure near the doors.

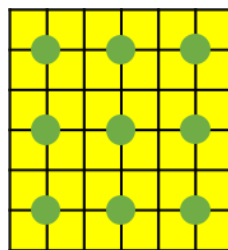
### 2.2.3.4 2012 Field trial design and protocol

Seed was sown by Simon Crawford of Burpee on the 16<sup>th</sup> of April, and ten-week old plants were planted outdoors at Henfaes between the 29<sup>th</sup> of June and 1<sup>st</sup> of July 2012. In order to facilitate plant management, determinate and indeterminate plants were grown in two separate sections of the field (**Figure 2.2**). Each section functioned as a separate fully randomised trial. Plants were spaced 90 cm apart each way on a square grid. Random positions within each section were generated by numbering each planting position and assigning trial plants to positions after ordering the plants according to random numbers generated using Microsoft Excel. In some cases, where fewer than 8 plants were available, the gap was filled using an arbitrarily chosen plant of another line. A guard-row of randomly positioned spare trial plants surrounded the field trial. The study site was somewhat heterogeneous in that the south-east end of the strip was more sheltered than the north-west, but this gradient was slight, and the distribution of lines was random along it. Weed suppressant membrane was laid on the soil surface. Soil nutrient status was deemed adequate, so no amendments were added. Soil moisture was at field capacity, and in places soil was waterlogged at times, owing to very heavy rainfall. Plants were watered heavily when they were first planted, but were not subsequently irrigated.



**Figure 2.2: Layout plan of field trials**

a. 2011, b. 2012, c. 2013, d. 2014. Red arrow in upper right of each figure indicates North. In a., each rectangle represents a group of three plants. In b.-d. each rectangle represents an individual plant. In c. and d., replicated blocks are indicated by alternating colour. In b. and c., a guard row of randomised spare trial plants surrounded the perimeter of the trial (not shown).



**Figure 2.3: Placement of spreader plants**

Spreader plants are indicated by green circles between trial plants (yellow squares) in the 2012 and 2013 trials.

The trial was inoculated with material taken from a nearby potato trial which had been inoculated with a 13\_A2 *P. infestans* isolate (David Shaw, SRT, *pers. comm.*). Spore suspension was prepared by washing spores from infected potato foliage using tap water. The sporangia concentration was not determined, but was sufficient to cause heavy infection on susceptible plants. The spore suspension was then applied to mature pot-grown susceptible spreader plants (cultivar “Roma”) on the 6<sup>th</sup> of September. The spreader plants were kept in a humid greenhouse for four days after inoculation and then distributed amongst the trial plants once they began to exhibit actively sporulating lesions (on the 10<sup>th</sup> of September). A spreader plant was placed between every second plant and its neighbour, such that there was one spreader plant to every four trial plants (**Figure 2.3**). This arrangement ensured that all trial plants were adjacent to one spreader plant. The perimeter guard row plants were treated as “trial plants” for purposes of laying out spreader plants. Owing to prevailing cold, dry weather, infection had not spread to the trial plants by the time the spreader plants had been largely defoliated, so the decision was taken to inoculate the trial plants directly (in the same manner as the spreader plants) on the 22<sup>nd</sup> of September. Inoculum was applied shortly before nightfall on a warm, humid evening.

#### 2.2.3.5 2013 Field trial design and protocol

The 2013 trial was set up as a complete randomised block design with one plant of each tomato line in each block. There were eight blocks in total, spread along the long axis of the trial site (**Figure 2.2**). Within each block, the distribution of plants was random. The random layout was generated using Microsoft Excel as described in *2012 Trial design and protocol*. Where fewer than eight plants of a line were available, priority was given to including plants in the two outermost blocks and two innermost blocks. Gaps left by missing plants were filled with arbitrarily chosen plants of other lines, meaning that the trial included more than eight plants of some lines (see raw data in **Supplementary materials**).

As in 2012, mature pot-grown spreader plants (cultivar “Ailsa Craig”) were inoculated with a 13\_A2 *P. infestans* strain and placed in the field in a similar manner to in 2012 (**Figure 2.3**).

#### 2.2.3.6 2014 Field trial design and protocol

The 2014 field trial consisted of 23 inbred lines developed during the course of this project, four commercial cultivars for comparison and four families of F<sub>2</sub> plants for blight resistance evaluation with a view to their possible use in future breeding work by Burpee.

The F<sub>2</sub> families were assessed by Simon Crawford and are not discussed further here. There were six plants of most of the inbred lines (see raw data in **Supplementary materials**). These were divided into two groups of three pseudo-replicates. One block of these groups was randomised

(as in 2012) whilst in the other block, the groups were planted in numerical order to facilitate discussing the lines at a commercial promotion day. The field trial was directly spray-inoculated (as in 2012) on the 25<sup>th</sup> of August and fruit and foliage disease severity was assessed on the 9<sup>th</sup> and 28<sup>th</sup> of October.

#### 2.2.4 Disease severity assessment

Frequent, *ad-hoc* monitoring on the field trials was commenced as soon as a *P. infestans* was reported in the local area (in all years, outbreaks occurred at the Llanfairfechan allotment site, approximately 3 km north-east of the trial site, before inoculation). Formal assessments of the field trial commenced once *P. infestans* infection was detected on trial plants, and both foliar and fruit blight were recorded separately. If the plant had one or more fruits  $\geq 15$  mm in diameter present then the fruit blight was estimated as proportion of individual fruits with lesions and recorded as a percentage:

$$\text{Fruit Blight} = 100 \times \frac{\text{Number of infected fruits}}{\text{Total number of fruits}}$$

A missing value was recorded if there were no fruits  $\geq 15$  mm in diameter. In the 2011 and 2014 trials which included groups of plants, each plant in a group was assessed individually, giving three pseudoreplicate scores for each line.

Foliage blight was assessed as the proportion of foliage visibly occupied by *P. infestans* lesions (i.e., brown/black/wilted/sporulating leaf regions) or already destroyed by *P. infestans* (dead leaves still attached to the plant stem, or bare stem regions where all leaves had died and detached).

$$\text{Foliage Blight} = 100 \times \frac{\text{Area of infected, dead \& missing foliage}}{\text{Total foliage area}}$$

#### 2.2.5 Mildew assessment

Many plants in the 2011 and 2012 trials were found to suffer from powdery mildew (primarily caused by *Oidium neolycopersici*, or *Leveillula taurica* (Kole 2007)). Whilst the focus of the studies conducted here was to evaluate *P. infestans* resistance, a simple assessment of powdery mildew was conducted in 2013. Each plant was examined on the 25<sup>th</sup> of August 2013 and given a rating of 0 (no visible infection), 1 (very slight infection, <5% foliage covered area by lesions), 2 (light infection, 6-10% foliage area covered), 3 (medium infection, 10-20% foliage area covered), 4

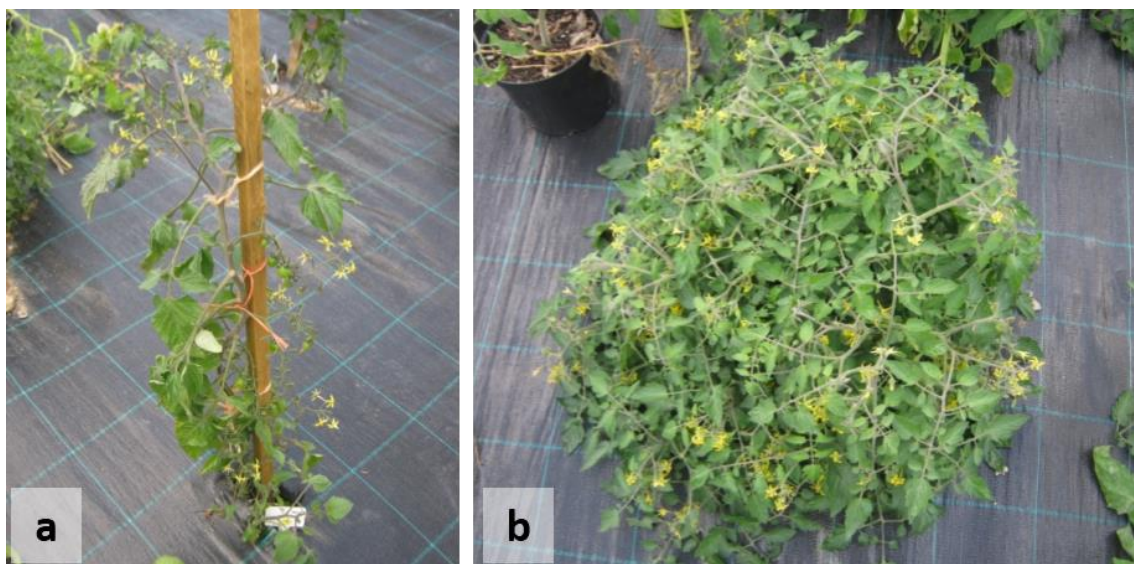
(heavy infection, 30-40% foliage area covered) of 5 (very heavy infection, >50% foliage area covered). A mean score was calculated for each tomato line.

### 2.2.6 2011 Polyunnel trial assessment

The first two visual blight assessments in the polyunnel trial (made on the 14<sup>th</sup> and 16<sup>th</sup> of August) considered the fruits and foliage together to give a whole-plant score on a 0-10 disease severity scale, with 0 indicating no visible infection, 1-9 quantifying (subjectively) increasing severity of infection, and 10 indicating complete defoliation and infection of all fruits. A single score was given for the average health of the group of plants (usually three plants). However, from the 28<sup>th</sup> of August onwards, fruits and foliage were assessed separately, and recorded for individual plants, as opposed to groups. From the 28<sup>th</sup> of August onwards, the percentage of foliage infected with or destroyed by blight was estimated visually as for the field trial, although when assessing the polyunnel trial, the disease severity on foliage was recorded to the nearest 10% only.

### 2.2.7 2012 Foliage density

Lines were given a ranking for foliage density on a 1 to 10 scale, with 1 initially being assigned to the Hazelnoot Tomaat, which had the most open structure, and 10 assigned to 11117A, which had the densest foliage (**Figure 2.4**). Other lines were given intermediate ranks relative to these extremes, based on an approximate visual assessment of the relative amount of open air versus leaves/stems within the space occupied by the plant.



**Figure 2.4: Examples of tomato plants with different foliage densities.**

a: Hazelnoot Tomaat, the line with the least dense foliage, assigned to rank 1; b: 11117C, the line with the densest foliage, assigned to rank 10.

## 2.2.8 *Ph-2* and *Ph-3* Genotyping

### 2.2.8.1 DNA Extraction

Unexpanded, healthy leaflets were collected from mature plants grown in the 2012 and 2013 field trials, placed in paper bags, and freeze-dried using an Edwards Modulyo K4 freeze-dryer and RV5 vacuum pump (Thermo-Fisher Scientific, Renfrew, Renfrewshire). Approximately 20 mg of freeze dried leaf tissue was ground in a microfuge tube using a Qiagen Tissue Lyser beadmill (Qiagen, Crawley, Sussex) with 8 mm steel balls in 1.5 mL Eppendorf Safelock tubes (Eppendorf Ltd, Stevenage, Hertfordshire). DNA extraction from the ground leaflet samples was carried out using a Qiagen DNEasy Plant Mini Kit (Qiagen, Crawley, Sussex) according to the manufacturer's instructions. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA). DNA was diluted to 20 ng  $\mu\text{L}^{-1}$  in Qiagen Buffer AE extraction buffer (Qiagen, Crawley, Sussex) and stored at  $-20\text{ }^{\circ}\text{C}$ .

### 2.2.8.2 Marker Selection

CAPS markers reviewed by Panthee and Foolad (2012) were used to screen for *Ph-2* and *Ph-3* *P. infestans* resistance genes (**Table 2.2**).

### 2.2.8.3 PCR Amplification

PCR Amplification was carried out using Bioline MyTaq PCR Mixture (Bioline, Taunton, Massachusetts, USA). The reaction mixture was prepared in 25  $\mu\text{L}$  total volume according to the manufacturer's instructions, using approximately 20 ng of template DNA and 20  $\mu$  moles of each primer. PCR conditions were as follows: 60 s at  $95\text{ }^{\circ}\text{C}$ , followed by 35 cycles of 15 s at  $95\text{ }^{\circ}\text{C}$ , 15 s at  $55\text{ }^{\circ}\text{C}$ , 10 s at  $72\text{ }^{\circ}\text{C}$ . PCR was carried out using a PTC-100 Thermocycler (Bio-Rad Laboratories Ltd., Hemel Hempstead, Hertfordshire).

### 2.2.8.4 Restriction Enzyme Digestion

Following PCR amplification, PCR products were digested with the appropriate restriction enzymes (**Table 2.2**). The following mixture was incubated: 6  $\mu\text{L}$  of water, 1  $\mu\text{L}$  of 10 x the appropriate digestion buffer (Thermo-Fisher Scientific, Renfrew, Renfrewshire), 1  $\mu\text{L}$  of restriction enzyme, and 10  $\mu\text{L}$  of PCR product. Incubation was at  $37\text{ }^{\circ}\text{C}$  for 4 h, using a PTC-100 Thermocycler (Bio-Rad Laboratories Ltd., Hemel Hempstead, Hertfordshire).

**Table 2.2: Details of *P. infestans* resistance gene CAPS markers tested.**

All markers are from Panthee and Foolad (2012). *Used* indicates whether the marker was selected for genotyping trial material, following testing of the markers with a small selection of DNA from tomato lines with known genotypes.

Gene	Marker	Restriction Enzyme <sup>a</sup>	Primer Sequences	Size of expected Digestion Products <sup>b</sup>	Used
<i>Ph-2</i>	dTG63	<i>Hinf</i> I	(F) CTACTCTTTCTATGCAATTTGAATTG (R) AATAATTTTCAACCATAGAATGATT	221 bp (S) 245 bp (R)	Yes
	dTG422	<i>Hinf</i> I	(F) TGACATGAGAAGGAAAAGACTTAAG (R) GTCAATAATTTTCAACCATAGAATGATT	290 bp (S) 310 bp (R)	Yes
<i>Ph-3</i>	TG328	<i>Bst</i> N1	(F) GGTGATCTGCTTATAGACTTGGG (R) AAGGTCTAAAGAAGGCTGGTGC	500 bp (S) 240 + 260 bp (R)	Yes
	dTG328	<i>Bst</i> N1	(F) GAATCTTCCCCTCGACCCCTCCTCACG (R) GGCTTGCATCTTTCTCCCCTTAAATACCAGC	320 bp (S) 170 + 150 bp (R)	No
	dTG328- F2/R2	<i>Bst</i> N1 or <i>Apo</i> I	(F) CCAGCTAACCAAATAACTATATGTAT (R) GGCTTGCATCTTTCTCCCCTTAAATACCAAA	400 (S) 250 + 150 (R)	Yes
	TG591	<i>Acl</i> I	(F) AAGGCAAAGGAAGTTGGAGGTCA (R) AGAGGTTGCAACTCGTGGATTGAG	150 bp (S) 160 bp (R) 400 bp (ND)	No
TG591- New	<i>Msp</i> I	(F) AAGGCAAAGGAAGTTGGAGGTCA (R) TGGCTATGTTAAGCAAGATTTCTC	150 bp (S) 160 bp (R) 400 bp (ND)	Yes	

<sup>a</sup> Restriction enzymes from Thermo Scientific (Thermo-Fisher Scientific, Renfrew, Renfrewshire). In a correction to (Foolad and Panthee 2012) (sic.), which also described the markers listed here, the authors advised that the restriction enzymes specified in Foolad and Panthee (2012) may give anomalous results and alternative enzymes should be used (Foolad and Panthee 2013). Unfortunately, most genotyping work had been completed when this information came to light. However, genotyping of the 2013 lines was repeated with *Apo*I, the correct enzyme.

<sup>b</sup> S = Susceptible allele, R = Resistant Allele, ND = Non Diagnostic band present for both alleles.

All markers described in Panthee and Foolad (2012) were trialled with DNA from seven tomato lines, including several with known *Ph-2* and *Ph-3* genotypes (Table 2.3). The markers which gave clearest results were then chosen to analyse the 2012 and 2013 tomato trial lines (Table 2.2)

**Table 2.3: Tomato lines used to test *Ph-2* and *Ph-3* markers.**

*Ph-2* and *Ph-3* indicate the genotype of each line where this could be independently verified. *Hom. Sus.* indicates homozygous susceptible, *Het.* heterozygous resistant, and *Hom. Res.* homozygous resistant.

Tomato Line	<i>Ph-2</i>	<i>Ph-3</i>	Genotype Info Source
Ailsa Craig <sup>a</sup>	-	-	-
NC2 Grape	Hom. Sus.	Hom. Sus.	Gardner and Panthee (2009)
Mountain Magic	Het.	Het.	Gardner (2008)
Red Alert	Hom. Sus.	Hom. Sus.	Simon Crawford ( <i>Pers. Comm.</i> )
NC2-CELBR	Hom. Res.	Hom. Res.	Gardner and Panthee (2010b)
Koralik	-	-	-
NC2-CELBR x Koralik	-	-	-

<sup>a</sup> Ailsa Craig is well established as a *P. infestans* susceptible variety in grey literature.

#### 2.2.8.5 Separation of restriction fragments

Separation method varied by marker. In the case of dTG63 and dTG422, digested PCR Products were visualised using 2% superfine resolution agarose (Amresco LLC, Solon, Ohio, USA) gel made with 1 x TAE Buffer (4.84 g Tris Base, 1.24 mL Acetic Acid, 200 mL 500 mM EDTA (pH 8) per litre), plus 15 µL per 100 mL of Safeview nucleic acid stain (NBS Biologicals Ltd., Huntingdon, Cambridgeshire). In the case of dTG328, a 2% agarose (Bioline, Greenwich, London) gel made with 1 x TBE buffer (10.8 g Tris Base, 5.5g Boric Acid, 4 mL 500 mM EDTA (pH 8) per litre) plus 15 µL per 100 mL of Safeview nucleic acid stain (NBS Biologicals Ltd., Huntingdon, Cambridgeshire) was used.

Gels (approximately 5 mm thick) were cast in a 20 x 25 cm in a Kodak Biomax 2025 gel tank (Kodak Ltd., Hemel Hempstead, Hertfordshire) and run at 110 V for one to two hours. Wells (1 x 3 mm and approximately 4 mm deep) were loaded with 8 µL of digested PCR product plus 2 µL of Bioline Crystal loading buffer (Bioline, Greenwich, London).

Visualisation was carried out using a UV2 transilluminator (Ultra-Violet Products Ltd., Cambridge, Cambridgeshire). Fragment sizes were estimated by comparison with either Bioline EasyLadder I DNA ladder or Bioline HyperLadder 25bp (Bioline, Greenwich, London).

For marker TG591-New, separation on agarose gels proved impossible because of the small size difference between the R and S alleles, and the low signal to noise ratio as a result of weak amplification of the diagnostic band. Accordingly, this marker was separated and visualised using



a Qiagen QIAxcel Advanced capillary electrophoresis system (Qiagen, Crawley, Sussex). The High Resolution capillary kit was used with Qiagen QX Alignment Marker 15 bp/600 bp and Qiagen QX DNA Size Marker 25 bp – 500 bp. 10 µL of digested PCR product was loaded according to the manufacturer's instructions, and run using the OM400 Method.

#### 2.2.8.6 Interpretation of marker data

Where a discrepancy between different markers was evident, in most cases a “best consensus” genotype was decided based upon the ease with which different markers could be scored accurately (i.e., giving greater weight to results from easily scorable markers compared to those which amplified poorly or had similar-sized R and S alleles). This consensus was further informed by the field performance of the genotype; in the case of highly resistant lines, it was usually presumed that a *Ph-* gene was present where the marker data were ambiguous (and vice versa for highly susceptible lines). The 2012 germplasm collection was genotyped at the end of the project, and owing to time constraints, only one of the selected pair of markers was used, and the assay was not repeated. Accordingly, the 2012 genotypes should be treated with caution.

#### 2.2.8.7 Use in Breeding Programme

Following data collection and analysis, reports on the field trials in each year were submitted to Simon Crawford of Burpee and (in 2011) John Burrows and Barrie Smith of Pro-Veg. The information was used to guide the Burpee and Pro-Veg tomato breeding programmes.

Since the F<sub>1</sub> hybrid cultivar Mountain Magic exhibited strong blight resistance and favourable horticultural characteristics in 2011 (see **Results**), a number of inbred lines were developed from it by Simon Crawford during the subsequent winter, and these were included in subsequent field trials to further evaluate their potential for use in Burpee's breeding programme.

### 2.2.9 Data Analysis

#### 2.2.9.1 Calculating disease severity

For each individual plant, the area under disease progress curve (AUDPC) was calculated using the following equation:

$$\text{AUDPC} = \sum \left( \frac{(S_i + S_{i+1})}{2} \times (D_{i+1} - D_i) \right)$$

where  $S_i$  = the disease severity (%) on the  $i^{\text{th}}$  assessment date, and  $S_{i+1}$  = the disease severity on the subsequent assessment date, and  $D_i$  = the number of days post inoculation on the  $i^{\text{th}}$  assessment day and  $D_{i+1}$  = the number of days post inoculation on the subsequent assessment day.

The calculation was carried out using Microsoft Excel. The statistics software SPSS 22 was used to calculate the mean AUDPC and 95% confidence for each line, which were plotted as error bar graphs. Trials were assessed on two to five dates in different years (**Table 2.1**) although for a small number of plants in some years, fewer assessments were made (See raw data in **Supplementary materials**). For replicates where more than half of the assessment dates were missing (because of loss of plants due to wind damage, for example), the replicate in question was excluded from further analyses.

Field and polytunnel datasets from 2011 were combined as follows; the AUDPC values were standardised by dividing each tomato line mean AUDPC value by the mean AUDPC for the whole trial (either field or polytunnel):

$$SAUDPC_g = \frac{AUDPC_g}{AUDPC_D}$$

where  $SAUDPC_g$  is the standardised AUDPC for tomato line  $g$ ,  $AUDPC_g$  is the AUDPC for tomato line  $g$ , and  $AUDPC_D$  is the mean of all AUDPC values for the dataset (either field or polytunnel). The SAUDPCs of both the polytunnel and field datasets were then used to produce the combined dataset by calculating a mean SAUDPC for each line.

The analysis for fruit scores in 2011 was based on 38 lines with sufficient fruits from the polytunnel only.

#### 2.2.9.2 *Effect of Ph- genotype on disease severity*

Two-way ANOVA was performed to examine the effect of *Ph-* genotype on the 2012 and 2013 fruit and foliage disease severity. The analysis was conducted in Minitab 17. Fruit and Foliage AUDPCs from both years were the response variables in four separate analyses, with *Ph-2* and *Ph-3* genotype as factors. Tukey's test was used for post-hoc multiple comparisons.

#### 2.2.9.3 *Comparison of SL11- families*

The 39 "SL11-" lines in the 2011 trial all belonged to four groups of sibling lines from different parental combinations. Kruskal-Wallis tests and Dunn Tests were carried out to identify which families differed significantly from each other in order to make inferences as to the contribution of different parents to *P. infestans* resistance.

#### 2.2.9.4 *Correlation between foliage and fruit disease severity*

Pearson's correlation between fruit and foliage disease severity (AUDPCs) was calculated using Minitab 17. The test was performed on all trials except the 2011 Field trial, for which no fruit

disease severity data was collected, and the 2014 Field Trial, in which fruit data was collected but this and foliage data contained many missing values.

#### 2.2.9.5 Correlation between foliage density and disease severity

Spearman's Rank Correlation between foliage AUDPC and foliage density class was calculated the, 2012 field trial data.

## 2.3 Results

### 2.3.1 Data collected

Mean performance of all tomato lines trialled in the four years of the project is presented in **Table 2.5** to **Table 2.8**, and **Figure 2.5** to **Figure 2.13**. **Table 2.4** lists all tomato lines which were among the best performing (most resistant) 10 lines in each trial, with the exception of the 2011 trial based on fruit AUDPC, as 95% confidence intervals were so wide as to make rankings of very little value.

The *Ph-2* and *Ph-3* genotypes of most lines grown in 2012 and 2013 are presented in **Table 2.6** and **Table 2.7**. Marker TG591 did not amplify well (not shown), and diagnostic bands were not visible. Marker TG591-N produced extremely weak diagnostic bands, which were not visible on an agarose gel, but could easily be detected with the QIAxcel Advanced capillary electrophoresis platform. TG328, dTG328 and dTG328-F2/R2 all produced clear, easily identifiable fragments following digestion. Marker dTG328-F2/R2 was adopted as the marker of choice owing to its ease of scoring and the low cost of the *BstN1* restriction enzyme, although *Ph-3* genotypes were subsequently retested using dTG328-F2/R2 and the *ApoI* restriction enzyme, following publication of a correction to the original source paper (Foolad and Panthee 2013). Genotyping of the 2012 tomato lines (which was conducted after genotyping of the 2013 lines) used the TG328 marker and *BstN1* enzyme. Both *Ph-2* markers proved difficult to score, owing to the similar size of the R and S fragments, and the fact that the markers amplified weakly, and accordingly the results presented here should be treated with caution (**Table 2.6** and **Table 2.7**).

### 2.3.2 Outstanding Lines

**Table 2.4** lists the ten best performing lines with regard to fruit blight resistance and foliage blight resistance, in 2011-2013. The fruit data from the 2011 polytunnel trial exhibited very wide confidence intervals (**Figure 2.7**) and a large number of missing lines which were either not

planted in the polytunnel or failed to set fruit (**Table 2.5**) and so is not included in **Table 2.4**, however.

In 2011, many of the lines exhibiting strongest blight resistance (**Table 2.4**) also exhibited undesirable horticultural traits such as lateness (in the case of, for example, Schrapwell, Zauberberg Streiffen, Make My Day and Grungy in the Sky) or producing inedible fruit (Emperador). As well as strong fruit and foliage blight resistance, Mountain Magic ripened fruit reasonably early, and the fruit quality was good (*ad lib* observations – data not shown).

In 2012, Mountain Magic was again a top performer, along with Defiant, another line carrying both *Ph-2* and *Ph-3*. The 2012 trial included the wild-type “currant tomatoes” Wild Sweeties, Mexico Midget, Sweet Pea Currant, Gold Rush Currant, and Rote Murrel, all of which showed a high degree of blight resistance. However, these lines all had the disadvantage of an “undomesticated” plant habit (highly branched indeterminate habit with little or no apical dominance, and vigorous vegetative growth), and low yields of very small fruit (*ad lib* observations – data not shown).

In the 2013 trial, most of the best-performing lines were Mountain Magic inbred progeny lines developed by Burpee. The breeding line NC2-CELEBR and cultivar Iron Lady both showed excellent fruit blight resistance, although also very late maturing fruit, which had a poor flavour in the case of NC2-CELEBR (*ad lib* observations, data not shown). The wild-type heritage cultivar Matt’s Wild Cherry showed low fruit and foliage disease levels, and clearly did not carry *Ph-2*, although the *Ph-3* genotype was ambiguous (**Table 2.7**). Line 12073, developed by Burpee, is included in **Table 2.4** as it was noted for having good yields of particularly high quality fruit early in the season, in addition to good fruit and foliage blight resistance and acceptable powdery mildew tolerance.

### 2.3.3 Powdery mildew susceptibility

Although there were individual plants from most lines with no visible powdery mildew infection (See raw data in **Supplementary materials**), no tomato line was completely immune. Cultivar Jasper showed the lowest incidence of mildew, with a mean score of 0.25. Matt’s Wild Cherry performed very well against mildew in addition to late blight, with a mean score of 0.63. For both of these lines, no individual plant had a score higher than 1 (See raw data in **Supplementary materials**). Mountain Magic had a mean score of 1.13, with the Mountain Magic progeny lines ranging from 0.71 to 3.0, with a reasonably even distribution of scores throughout this range (**Table 2.7**).

**Table 2.4: Most resistant lines in all trials**

The 10 lines with lowest disease severity on fruit and foliage in all years.

<b>Trial</b>	<b>Line Name</b>	<b>Reason for selection</b>
2011 Field, Tunnel	Schrapwell	Foliage AUDPC
2011 Field	BL-10078	Foliage AUDPC
2011 Field, Tunnel	Make My Day	Foliage AUDPC
2011 Field & Tunnel	Mountain Magic	Foliage AUDPC
2011 Field, Tunnel	Skykomish	Foliage AUDPC
2011 Field, Tunnel	Emperador	Foliage AUDPC
2011 Field	SL11-49	Foliage AUDPC
2011 Field	Grungy in the Sky	Foliage AUDPC
2011 Field	West-Virginia 63	Foliage AUDPC
2011 Field	SL11-50	Foliage AUDPC
2011 Tunnel	Zauberberg Streiffen	Foliage AUDPC
2011 Tunnel	BL-10053	Foliage AUDPC
2011 Tunnel	10078	Foliage AUDPC
2011 Tunnel	Grungy in the Sky	Foliage AUDPC
2011 Tunnel	B-10058	Foliage AUDPC
2012 Field	Mountain Magic	Fruit and Foliage AUDPC
2012 Field	Rote Murrel	Fruit and Foliage AUDPC
2012 Field	Defiant	Foliage AUDPC
2012 Field	Wild Sweeties	Foliage AUDPC
2012 Field	Mexico Midget	Foliage AUDPC
2012 Field	Fandango	Foliage AUDPC
2012 Field	Ferline	Foliage AUDPC
2012 Field	PS150059	Foliage AUDPC
2012 Field	Fantasio	Foliage AUDPC
2012 Field	Sweet Hearts	Foliage AUDPC
2012 Field	Sweet Pea Currant	Fruit AUDPC
2012 Field	Gold Rush Currant	Fruit AUDPC
2012 Field	Sweet Canary	Fruit AUDPC
2012 Field	11117A	Fruit AUDPC
2012 Field	Wild Sweeties	Fruit AUDPC
2012 Field	1117B	Fruit AUDPC
2012 Field	Amai	Fruit AUDPC
2012 Field	Besjestomaat	Fruit AUDPC
2013 Field	MM21-2	Foliage and Fruit AUDPC
2013 Field	MM23-2	Foliage and Fruit AUDPC
2013 Field	MM45	Foliage AUDPC
2013 Field	MM21-1	Foliage AUDPC
2013 Field	MM6-2	Foliage AUDPC
2013 Field	Matt's Wild Cherry	Foliage and Fruit AUDPC
2013 Field	MM16-2	Foliage AUDPC
2013 Field	BN14-12020	Foliage AUDPC
2013 Field	MM16-1	Foliage AUDPC
2013 Field	BN14-12073	15 <sup>th</sup> Lowest Foliage AUDPC
2013 Field	MM45	Fruit AUDPC
2013 Field	MM6-2	Fruit AUDPC
2013 Field	MM23-1	Fruit AUDPC
2013 Field	MM29-3	Fruit AUDPC
2013 Field	NC2-CELEBR	Fruit AUDPC
2013 Field	Iron Lady	Fruit AUDPC

### 2.3.4 Tabulated disease severity and genotype data

**Table 2.5: 2011 trials disease severity**

Disease severity on the 2011 trials as standardised Areas Under Disease Progress Curve. All raw AUDPC values divided the mean AUDPC of all trials.

Line Name	Field Foliage		Tunnel Fruit		Tunnel Foliage		Line Name	Field Foliage		Tunnel Fruit		Tunnel Foliage	
	SAUDPC	N	SAUDPC	N	SAUDPC	N		SAUDPC	N	SAUDPC	N	SAUDPC	N
BL-10023	1.33	3		0	0.62	3	SL11-31	1.68	3	0.90	2	1.52	3
BL-10026	0.32	3		0	0.69	3	SL11-32	1.53	3	1.17	3	1.58	3
BL-10038	3.20	3		0	1.13	3	SL11-34	1.74	3	0.90	2	1.60	3
BL-10053	0.41	3		0	0.18	3	SL11-35	1.78	3	0.93	3	1.86	3
BL-10058	0.83	3		0	0.34	3	SL11-36	1.81	2	1.04	3	1.45	3
BL-10062		0		0	0.38	3	SL11-38	2.57	2	0.86	3	1.69	3
BL-10078	0.05	3		0	0.24	3	SL11-39	2.27	3	1.48	3	1.53	3
BL-1632209		0		0	1.20	3	SL11-40	1.42	3	1.00	3	1.43	3
BL-2122811	0.41	2		0		0	SL11-42	1.41	3	0.99	3	1.19	3
Emperador	0.11	2		0	0.17	3	SL11-49	0.14	3	1.20	2	0.93	3
Grungy in the Sky	0.21	2		0	0.26	3	SL11-50	0.27	3		0	0.66	3
Make My Day	0.05	3		0	0.13	3	SL11-51	0.48	3	0.86	2	1.43	3
Mountain Magic	0.06	6		0	0.15	6	SL11-52	0.54	3	1.46	3	1.24	3
Schrapwell	0.02	3		0	0.15	3	SL11-53	0.36	3	0.90	3	1.09	3
Skykomish	0.10	3		0	0.18	3	SL11-55	0.73	3	1.41	2	1.19	3
SL11-04	2.09	3	1.11	3	1.69	3	SL11-56	0.51	3	1.04	1	1.00	3
SL11-05	2.49	3	1.14	2	0.76	3	SL11-57	2.00	3	1.07	3	1.60	3
SL11-07	1.31	3	0.77	2	1.41	3	SL11-58	1.78	3	0.85	3	1.12	3
SL11-08	0.81	2	0.40	2	0.79	3	SL11-59	1.52	3	1.51	3	1.11	3
SL11-09	1.58	3	0.36	1	0.43	3	SL11-60	0.92	3	0.96	2	1.52	3
SL11-11	0.75	3	1.22	3	0.95	3	SL11-62	1.60	3	1.09	3	1.20	3
SL11-13	0.82	3	0.23	2	0.55	2	SL11-63	1.48	3	1.21	3	1.38	3
SL11-15	0.91	2	0.97	2	0.98	3	SL11-64	0.33	3	0.79	2	1.33	3
SL11-16	0.60	3		0		0	SL11-66	1.50	3	0.77	2	1.55	3
SL11-18	0.60	3	0.39	2	0.59	3	SL11-67	0.29	3	0.77	2	1.72	3
SL11-19	1.56	3	1.34	2	1.38	3	SL11-68	0.44	3	0.93	3	1.67	3
SL11-21	0.93	3	1.76	1	0.99	2	SL11-69	1.06	2	0.51	3	2.01	3
SL11-22	1.02	3	1.50	3	0.90	3	WV-63	0.24	3		0	0.51	3
SL11-27	1.29	3		0	0.83	3	Zberg. Streifen	0.62	3		0	0.15	2
SL11-28	1.06	3	0.57	3	1.13	3							

**Table 2.6: 2012 Field trial results and genotypes**

Name	Foliage		Fruit		Ph-2 genotype (dTG422) <sup>a</sup>	Ph-3 Genotype (TG328)
	AUDPC	N	AUDPC	N		
11118	1299	8	1148	5	<i>Sus/Sus</i>	<i>Ph-3/Ph-3</i>
11117A	1600	8	1288	7	<i>Sus/Sus</i>	<i>Sus./Sus.</i>
11117B	2967	7	1258	6	<i>Sus/Sus</i>	<i>Ph-3/Ph-3</i>
11117C	1880	8	1095	1	Failed	Failed
11118A	1974	8	1460	8	<i>Sus./Sus.</i>	<i>Ph-3/Ph-3</i>
Ailsa Craig	1749	8	1190	7	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
Amai	1655	8	1355	2	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
Bella Rosa	2443	7	1649	7	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
Besjestomaat	1666	8	2051	8	<i>Ph-2/Ph-2</i>	<i>Ph-3/Sus.</i>
Brione	1979	7	1351	7	<i>Ph-2/Ph-2</i>	<i>Sus./Sus.</i>
Defiant	521	8	3159	8	<i>Sus./Sus.</i>	<i>Ph-3/Ph-3</i>
Dutch Angelle	2055	7	1695	7	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Fandango	884	8	2491	8	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Fantasio	1129	7	2668	6	<i>Ph-2/Ph-2</i>	<i>Sus./Sus.</i>
Ferline	971	8	2511	8	<i>Sus./Sus.</i>	<i>Ph-3/Sus.</i>
Firebell	1313	8	1658	8	<i>Ph-2/Sus.</i>	<i>Ph-3/Ph-3</i>
Five Star Grape	1410	6	1584	3	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Giant Syrian	1745	8	2120	8	Failed	Failed
Gold Rush Currant	1199	7	1052	5	<i>Ph-2/Ph-2</i>	<i>Ph-3/Sus.</i>
Hazelnoot Tomaat	2061	7	2365	6	<i>Sus./Sus.</i>	<i>Sus./Sus.</i>
Indigo Rose	1783	8	1131	8	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
Japanese Black Truffle	2405	8	2558	8	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Matina	2018	8	1641	8	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
Mexico Midget	787	8	1839	8	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Mountain Magic	196	8	2214	8	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
Paprikatomaat Italien	1580	8	2157	8	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Previa	1276	8	2529	8	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
PS 150056	1667	8	2992	8	<i>Sus./Sus.</i>	<i>Sus./Sus.</i>
PS 150059	1105	7	2985	6	<i>Sus./Sus.</i>	<i>Sus./Sus.</i>
Pyros	1277	8	2383	8	<i>Ph-2/Ph-2</i>	<i>Sus./Sus.</i>
Red Alert	2777	8	2260	7	<i>Ph-2/Sus.</i>	<i>Sus./Sus.</i>
Rote Murrel	507	8	2178	7	<i>Ph-2/Ph-2</i>	<i>Sus./Sus.</i>
Silvery Fir Tree	2673	8	2525	8	<i>Sus./Sus.</i>	<i>Sus./Sus.</i>
Sixtina	1362	6	2428	6	<i>Ph-2/Sus.</i>	<i>Ph-3/Ph-3</i>
Sweet Canary	1639	8	2317	7	<i>Sus./Sus.</i>	<i>Sus./Sus.</i>
Sweet Hearts	1176	5	1364	5	Failed	Failed
Sweet Pea Currant	1420	6	1715	6	Failed	Failed
TMAC505	1892	6	1679	6	<i>Sus./Sus.</i>	<i>Ph-3/Ph-3</i>
Velvet Red	1874	5	1259	5	Failed	Failed
Wild Sweeties	689	6	1714	6	Failed	Failed

**Table 2.7: 2013 Field results and genotypes**

Mean AUDPCs for each tomato line, mean mildew severity on 28<sup>th</sup> of September (ranked 0-3, with 3 being the heaviest infection), *Ph-2* and *Ph-3* genotypes indicated by several markers, and the *Ph-* genotypes best supported by marker and phenotypic evidence (in the view of the authors).

Line Name	Fol. AUD-PC	Fruit AUD-PC	Mildew	N	<i>Ph-2<sup>a</sup></i>			<i>Ph-3<sup>a</sup></i>			Best <i>Ph-2<sup>b</sup></i>	Best <i>Ph-3<sup>b</sup></i>
					dTG 63 #1	dTG 63 #2	dTG 422	dTG328-F2/R2 <i>BstNI</i>	dTG328-F2/R2 <i>ApoI</i>	TG591-N <i>MspI</i>		
12005	786	900	1.45	11	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12009	796	1028	1.13	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12020	579	650	1.11	9	Failed	Het.	Het.	Het.	Het.	Failed	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12034	668	933	1.63	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12035	675	979	1.38	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12047	871	1015	1	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12061	703	1001	1.89	9	Res.	Res.	Res.	Het.	Het.	Het.	<i>Ph-2/Ph-2</i>	<i>Ph-3/Sus.</i>
12062	665	820	1.75	8	Het.	Het.	Het.	Het.	Het.	Res.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12071	630	1064	2.13	8	Het.	Het.	Het.	Het.	Het.	Res.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
12073	640	938	1.75	8	Res.	Res.	Res.	Res.	Het.	Res.	<i>Ph-2/Ph-2</i>	<i>Ph-3/Sus.</i>
12148	980	966	3.88	8	Res.	Res.	Res.	Sus.	Sus.	Sus.	<i>Ph-2/Ph-2</i>	Sus./Sus.
Ailsa Craig	1122	930	2.44	9	Sus.	Sus.	Sus.	Sus.	Sus.	Sus.	Sus./Sus.	Sus./Sus.
Early Sue	1026	1051	0.67	9	Res.	Res.	Res.	Sus.	Sus.	Sus.	<i>Ph-2/Ph-2</i>	Sus./Sus.
F2 (Rmn. Skies x Gn. Brandy)	1079	1117	1	5	Sus.	Sus.	Failed	Sus.	Sus.	Res.	Sus./Sus.	Sus./Sus.
Ferline <sup>c</sup>	1063	1074	1.86	7	Het.	Het.	Het.	Het.	Sus.	Het.	<i>Ph-2/Sus.</i>	Sus./Sus.
H12-30-1	917	1031	1.88	7	Sus.	Res.	Sus.	Sus.	Sus.	Het.	Sus./Sus.	Sus./Sus.
H12-30-2	1099	1089	2.75	8	Res.	Het.	Res.	Res.	Sus.	Res.	<i>Ph-2/Sus.</i>	Sus./Sus.
H12-30-3	1069	1005	1	8	Failed	Res.	Failed	Res.	Sus.	Res.	<i>Ph-2/Ph-2</i>	Sus./Sus.
H12-32-1	1063	1051	3.25	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
H12-32-2	1024	965	2.11	8	Het.	Het.	Het.	Het.	Het.	Het.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
H12-32-3	1060	1008	3.13	9	Sus.	Sus.	Sus.	Sus.	Sus.	Sus.	Sus./Sus.	Sus./Sus.
Iron Lady	1084	1066	2.33	8	Res.	Res.	Res.	Res.	Res.	Res.	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
Jasper	689	390	0.25	6	Sus.	Sus.	Sus.	Het.	Sus.	Res.	Sus./Sus.	<i>Ph-3/Sus.</i>



Continued overleaf

Table 2.7 continued

Line Name	Fol. AUD-PC	Fruit AUD-PC	Mild-ew	N	Ph-2 <sup>a</sup>			Ph-3 <sup>a</sup>			Best Ph-2 <sup>b</sup>	Best Ph-3 <sup>b</sup>
					dTG 63 #1	dTG 63 #2	dTG 422	dTG328-F2/R2 BstNI	dTG328-F2/R2 ApoI	TG591-N MspI		
Koralik	909	807	3.22	8	Res.	Res.	Res.	Sus.	Sus.	Sus.	Ph-2/Ph-2	Sus./Sus.
Koralik x NC2-CELBR F <sub>1</sub>	811	678	3.22	9	Res.	Res.	Res.	Het.	Het.	Het.	Ph-2/Ph-2	Ph-3/Sus.
Lieven Free	970	761	2.5	4	Sus.	Sus.	Sus.	Sus.	Sus.	Sus.	Sus./Sus.	Sus./Sus.
Losetto	1037	973	4.5	8	Het.	Het.	Het.	Het.	Sus.	Sus.	Ph-2/Sus.	Sus./Sus.
Magic Line-Up	901	883	0.88	8	Res.	Res.	Res.	Het.	Het.	Het.	Ph-2/Ph-2	Ph-3/Sus.
Make My Day	995	898	3.44	9	Het.	Het.	Het.	Res.	Sus.	Res.	Ph-2/Sus.	Sus./Sus.
Matt's Wild Cherry	484	139	0.63	8	Sus.	Sus.	Sus.	Sus.	Sus.	Res.	Sus./Sus.	Unknown
MM16-1 (F5) Ph2/Ph2	573	716	1.33	9	Res.	Res.	Res.	Res.	Failed	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM16-2 (F5) Ph2/Ph2	619	554	1.5	8	Res.	Res.	Res.	Res.	Failed	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM21-1 (F5) Ph2/Ph2	249	215	1	8	Res.	Res.	Res.	Res.	Res.	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM21-2 (F5) Ph2/Ph2	225	285	1	8	Res.	Res.	Res.	Res.	Res.	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM23-1 (F5) Ph2/Ph2	683	415	3	8	Res.	Res.	Res.	Res.	Res.	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM23-2 (F5) Ph2/Ph2	371	200	1.22	9	Res.	Res.	Res.	Res.	Res.	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM29-1 (F5) Ph2/Ph2	1010	950	1.75	8	Sus.	Sus.	Failed	Failed	Failed	Res.	Sus./Sus.	Unknown
MM29-2 (F5) Ph2/Ph2	1092	1074	3	8	Res.	Res.	Res.	Res.	Sus.	Res.	Ph-2/Ph-2	Sus./Sus.
MM29-3 (F5) Ph2/Ph2	494	298	2.38	8	Res.	Res.	Res.	Res.	Res.	Res.	Ph-2/Ph-2	Ph-3/Ph-3
MM30 (F4) Ph2/Ph2	864	1070	1.13	8	Res.	Res.	Res.	Res.	Sus.	Res.	Ph-2/Ph-2	Sus./Sus.
MM45 (F4) Ph2/Ph2	316	65	0.75	8	Sus.	Sus.	Sus.	Res.	Res.	Res.	Sus./Sus.	Ph-3/Ph-3
MM48-1 (F5) Ph2/Ph2	652	776	2.86	8	Sus.	Sus.	Het.	Res.	Res.	Res.	Unkn.	Ph-3/Ph-3
MM48-2 (F5) Ph2/Ph2	950	500	2.43	7	Sus.	Sus.	Het.	Res.	Res.	Res.	Unkn.	Ph-3/Ph-3
MM48-3 (F5) Ph2/Ph2	948	321	2	6	Sus.	Sus.	Sus.	Res.	Res.	Res.	Sus./Sus.	Ph-3/Ph-3
MM6-1 (F5) Ph2/Ph2	674	895	1.75	8	Res.	Res.	Res.	Res.	Sus.	Res.	Ph-2/Ph-2	Sus./Sus.
MM6-2 (F5) Ph2/Ph2	175	317	1.5	8	Het.	Het.	Het.	Res.	Res.	Res.	Ph-2/Sus.	Ph-3/Ph-3
MM63-1 (F5) Ph2/Ph2	854	873	0.71	7	Res.	Res.	Res.	Res.	Het.	Res.	Ph-2/Ph-2	Ph-3/Sus.
MM63-2 (F5) Ph2/Ph2	719	995	0.88	8	Res.	Res.	Res.	Res.	Het.	Res.	Ph-2/Ph-2	Ph-3/Sus.

Continued overleaf

Table 2.7 continued

Line Name	Fol. AUD-PC	Fruit AUD-PC	Mild-ew	N	<i>Ph-2<sup>a</sup></i>			<i>Ph-3<sup>a</sup></i>			Best <i>Ph-2<sup>b</sup></i>	Best <i>Ph-3<sup>b</sup></i>
					dTG 63 #1	dTG 63 #2	dTG 422	dTG328-F2/R2 <i>Bst</i> NI	dTG328-F2/R2 <i>Apo</i> I	TG591-N <i>Msp</i> I		
MM66-1 (F5) Ph2/Ph2	1028	1026	1.75	8	Res.	Res.	Res.	Res.	Sus.	Res.	<i>Ph-2/Ph-2</i>	Sus./Sus.
MM66-2 (F5) Ph2/Ph2	1022	929	1.44	9	Failed	Res.	Res.	Sus.	Sus.	Res.	<i>Ph-2/Ph-2</i>	Sus./Sus.
MM66-3 (F5) Ph2/Ph2	1081	1021	2.5	8	Sus.	Sus.	Sus.	Sus.	Sus.	Failed	Sus./Sus.	Sus./Sus.
MM70-1 (F5) Ph2/Ph2	944	1028	2.38	8	Res.	Res.	Res.	Res.	Sus.	Res.	<i>Ph-2/Ph-2</i>	Sus./Sus.
MM70-2 (F5) Ph2/Ph2	884	995	1	8	Res.	Res.	Res.	Res.	Sus.	Res.	<i>Ph-2/Ph-2</i>	Sus./Sus.
Mountain Magic	488	432	1.13	8	Het.	Het.	Het.	Res.	Het.	Res.	<i>Ph-2/Sus.</i>	<i>Ph-3/Sus.</i>
NC2 CELEBR	674	557	1.13	8	Res.	Res.	Res.	Res.	Res.	Res.	<i>Ph-2/Ph-2</i>	<i>Ph-3/Ph-3</i>
NC2 Grape <sup>c</sup>	1105	1189	0.88	8	Sus.	Sus.	Failed	Res.	Sus.	Res.	Sus./Sus.	Sus./Sus.
Omer-49	1056	1131	1.22	9	Sus.	Sus.	Sus.	Het.	Sus.	Het.	Sus./Sus.	Sus./Sus.
Omer-56	1071	961	1.88	8	Sus.	Sus.	Sus.	Res.	Sus.	Res.	Sus./Sus.	Unkn.
Omer-67	1085	995	0.63	8	Sus.	Sus.	Sus.	Het.	Sus.	Res.	Sus./Sus.	Sus./Sus.
Previa	1055	1029	0.75	8	Het.	Het.	Het.	Het.	Sus.	Het.	<i>Ph-2/Sus.</i>	Sus./Sus.
Red Alert	1010	1109	0.5	8	Sus.	Sus.	Sus.	Sus.	Sus.	Sus.	Sus./Sus.	Sus./Sus.
Rote Murrel	725	693	1.75	8	Sus.	Sus.	Sus.	Res.	Sus.	Res.	Sus./Sus.	<i>Ph-3/Ph-3</i>
Sky Reacher	1099	1048	1.17	6	Sus.	Het.	Het.	Sus.	Sus.	Sus.	<i>Ph-2/Sus.</i>	Sus./Sus.
Sky Reacher Tutt	1079	1062	2.25	4	Failed	Sus.	Sus.	Sus.	Sus.	Sus.	Sus./Sus.	Sus./Sus.
Skykomish	1057	993	3	6	Het.	Het.	Failed	Sus.	Sus.	Sus.	<i>Ph-2/Sus.</i>	Sus./Sus.
Trunnel Stake	1117	1076	2.8	5	Failed	Het.	Het.	Sus.	Sus.	Sus.	<i>Ph-2/Sus.</i>	Sus./Sus.

<sup>a</sup> Marker genotypes are indicated as Sus. where they are homozygous for the susceptible-linked allele, Res. when they are homozygous for the resistant-linked allele, and Het. when they carry a copy of each allele. Failed indicates that the fragment was either not amplified, or could not be unambiguously identified.

<sup>b</sup> The symbol + indicates the absence of a resistance gene.

<sup>c</sup> NC2-Grape and Ferline do not carry a functioning *Ph-3* resistance gene, but this study indicated that they do carry the Res allele of the TG591-N marker, and the dTG328-F2/R2 marker when the digestion is performed with *Bst*NI

**Table 2.8: 2014 Field Trial AUDPCs**

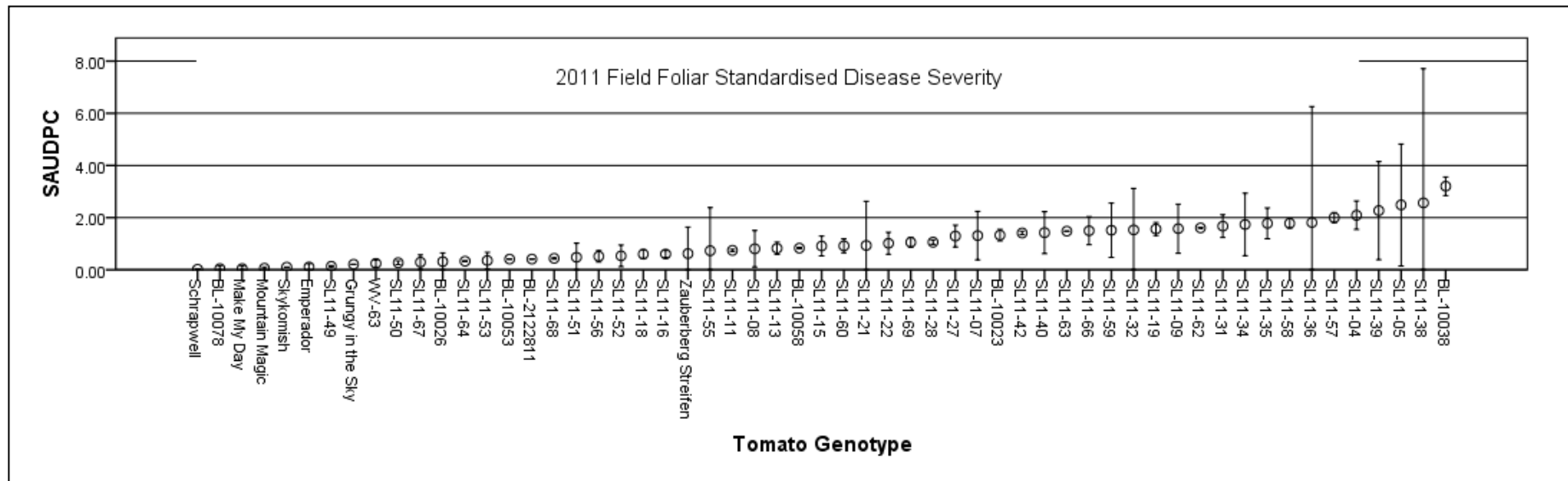
Line	Fruit Mean AUDPC	N	Foliage Mean AUDPC	N
12008	1350	3	1750	3
12073	227	3	243	3
13002	433	2	675	2
13012	718	2	1063	2
13020	1054	4	1332	3
13026	1127	6	561	3
13027	48	6	243	3
13028	1502	6	322	3
13077	393	4	276	3
13078	582	5	564	3
13185	2710	5	2517	6
13235	392	6	1312	6
13236	50	6	675	3
13241	1692	6	1620	3
13244	227	6	2403	6
13246	1254	6		0
13249	48	5	923	5
13252	1379	3	1350	2
13257	1485	3	1050	1
13259	636	5	422	3
13260	236	4	68	2
13261	104	5	641	5
13262	29	5	44	5
12003A	2903	6	1950	1
Fandango		0		0
Fantasio		0		0
Ferline	1662	3		0
Mountain Magic	109	5	860	6

### 2.3.5 Performance of material in 2014 trial

The 2014 plantings did not display substantial *P. infestans* infection until early October by which time the plants were beginning to senesce, making late-blight assessment more difficult. Ferline, Fantasio and Fandango had senesced to the point that assessing late blight severity was impossible. However, Mountain Magic plants were still healthy, as were plants of a number of the new lines being assessed, well into October. Approximately 50% of the new lines being assessed had foliage AUDPCs less than or equal to Mountain Magic (**Table 2.8; Figure 2.12**). A similar number of lines also had fruit blight AUDPCs similar to that of Mountain Magic (**Table 2.8; Figure 2.13**).

### 2.3.6 Error bar graphs of all trial data

The following figures (**Figure 2.5 to Figure 2.13**) show the mean disease severity on both foliage and fruit for each field trial, along with the 95% confidence intervals



**Figure 2.5: 2011 field standardised foliage disease severity.**

Mean standardised AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.

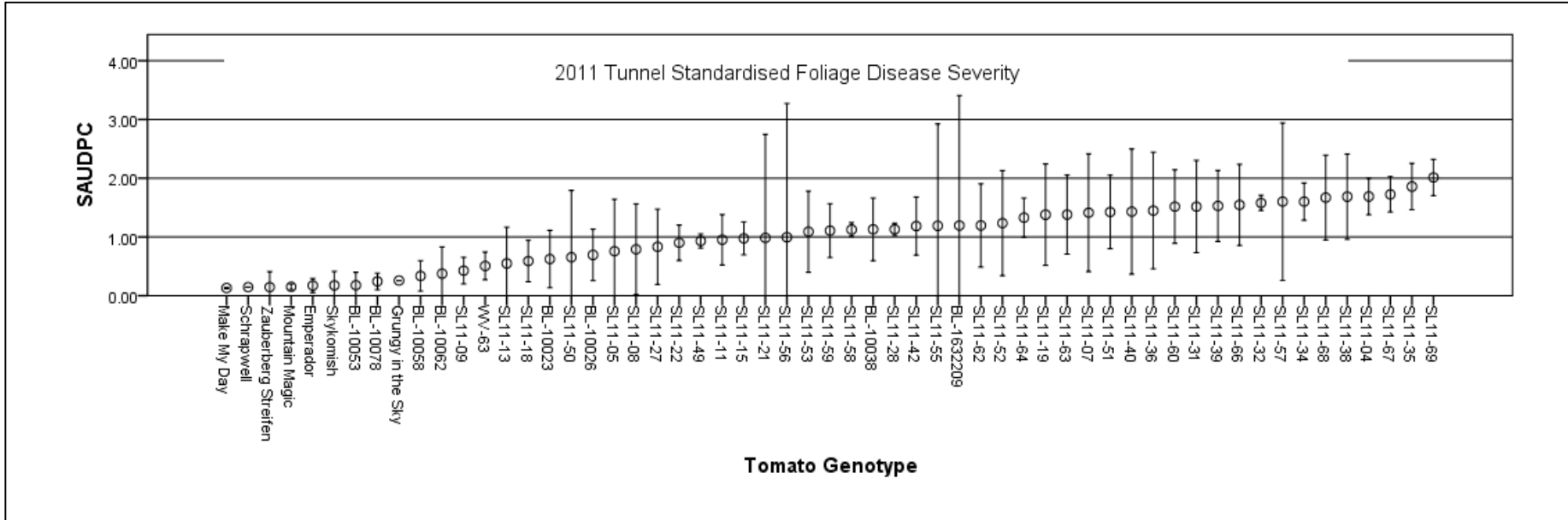
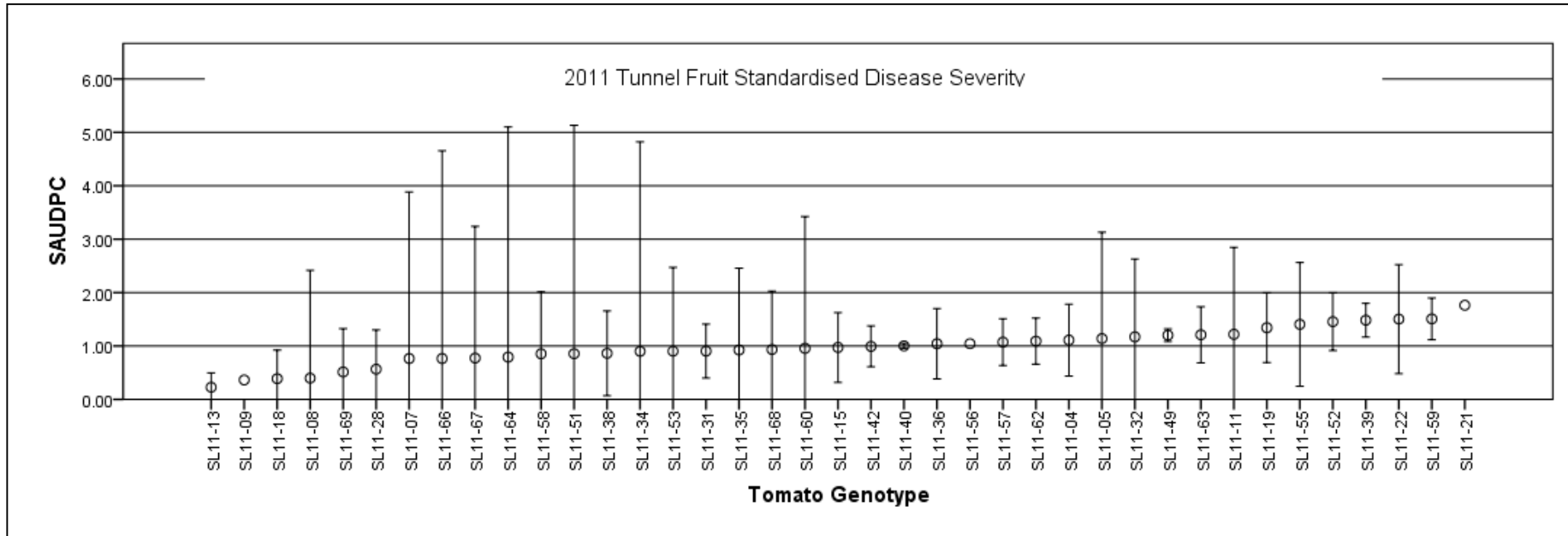


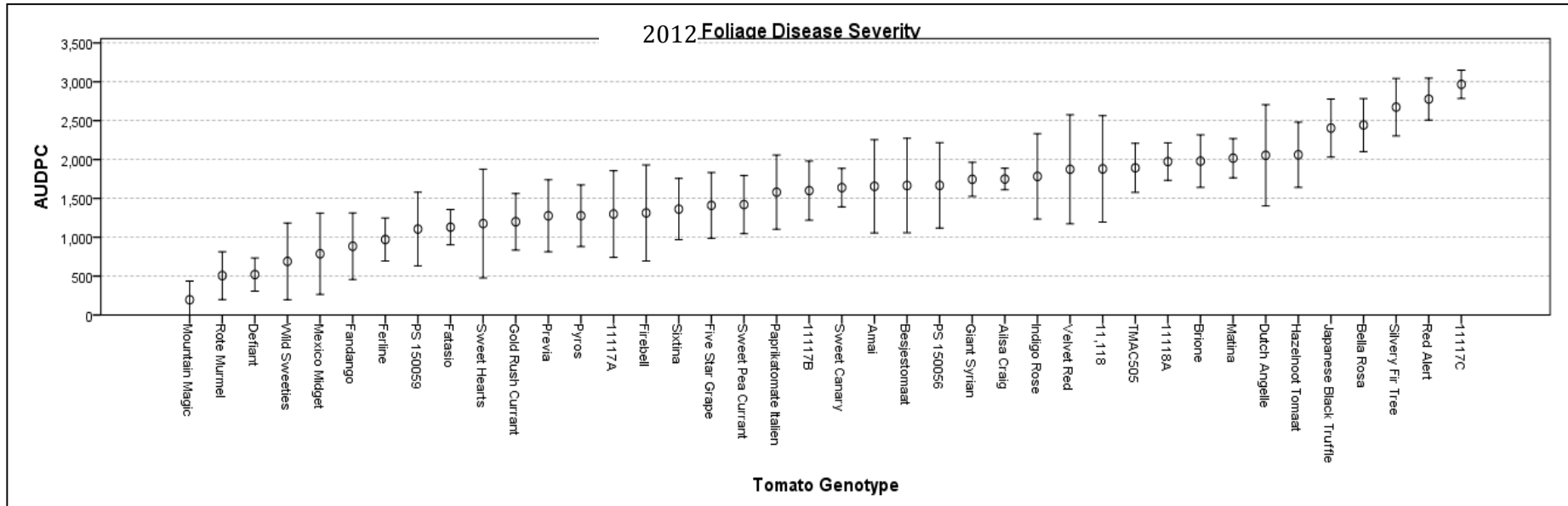
Figure 2.6: 2011 tunnel standardised foliage disease severity.

Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.



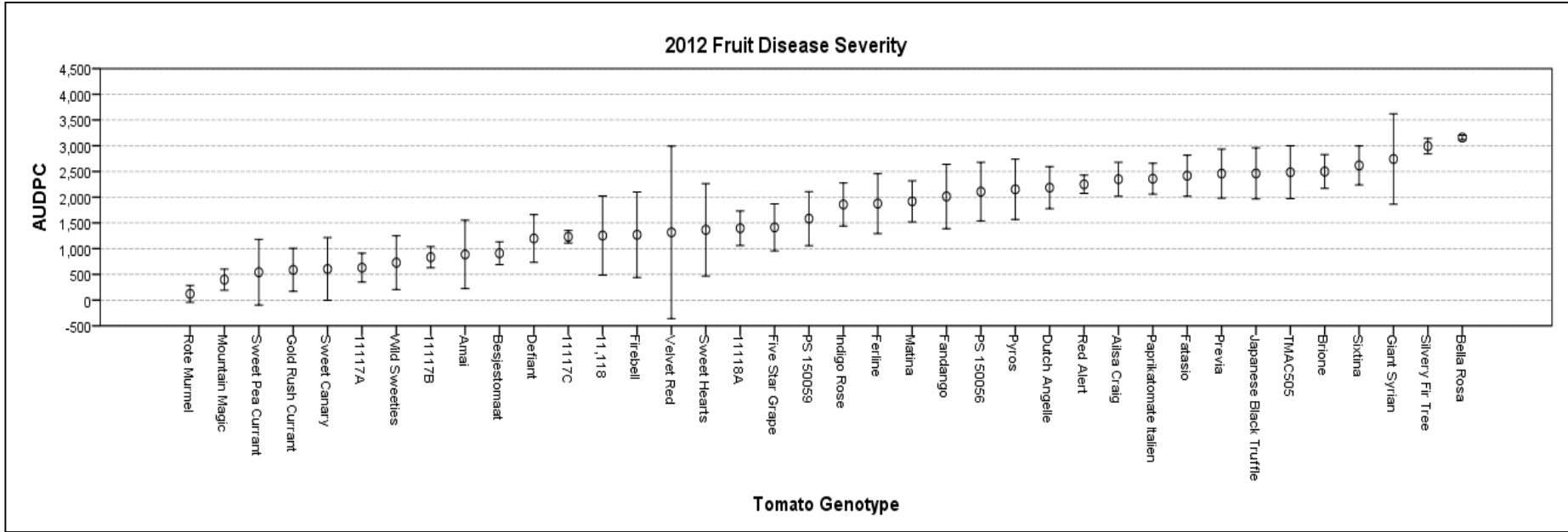
**Figure 2.7: 2011 tunnel fruit disease severity.**

Mean AUDPC for all tomato lines in the polytunnel trial which produced fruit, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.



**Figure 2.8: 2012 foliage disease severity.**

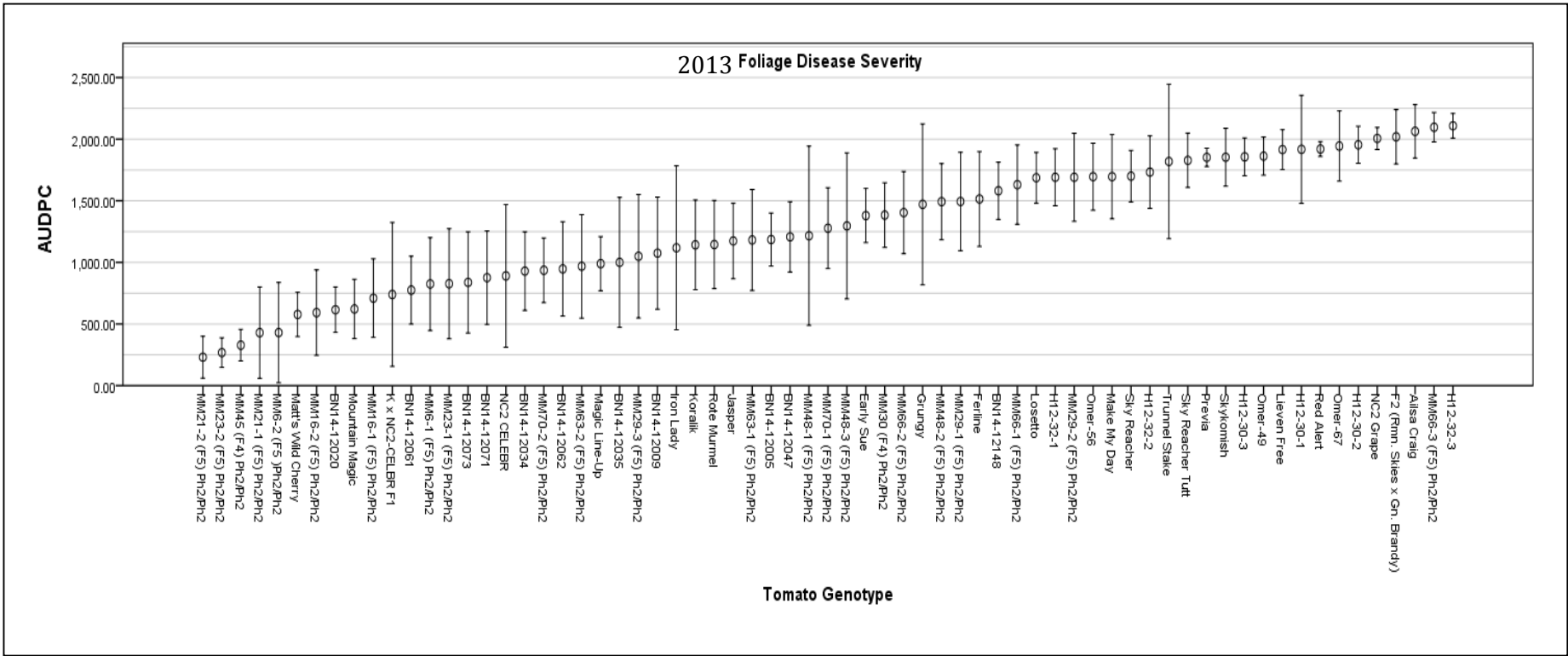
Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.



**Figure 2.9: 2012 fruit disease severity.**

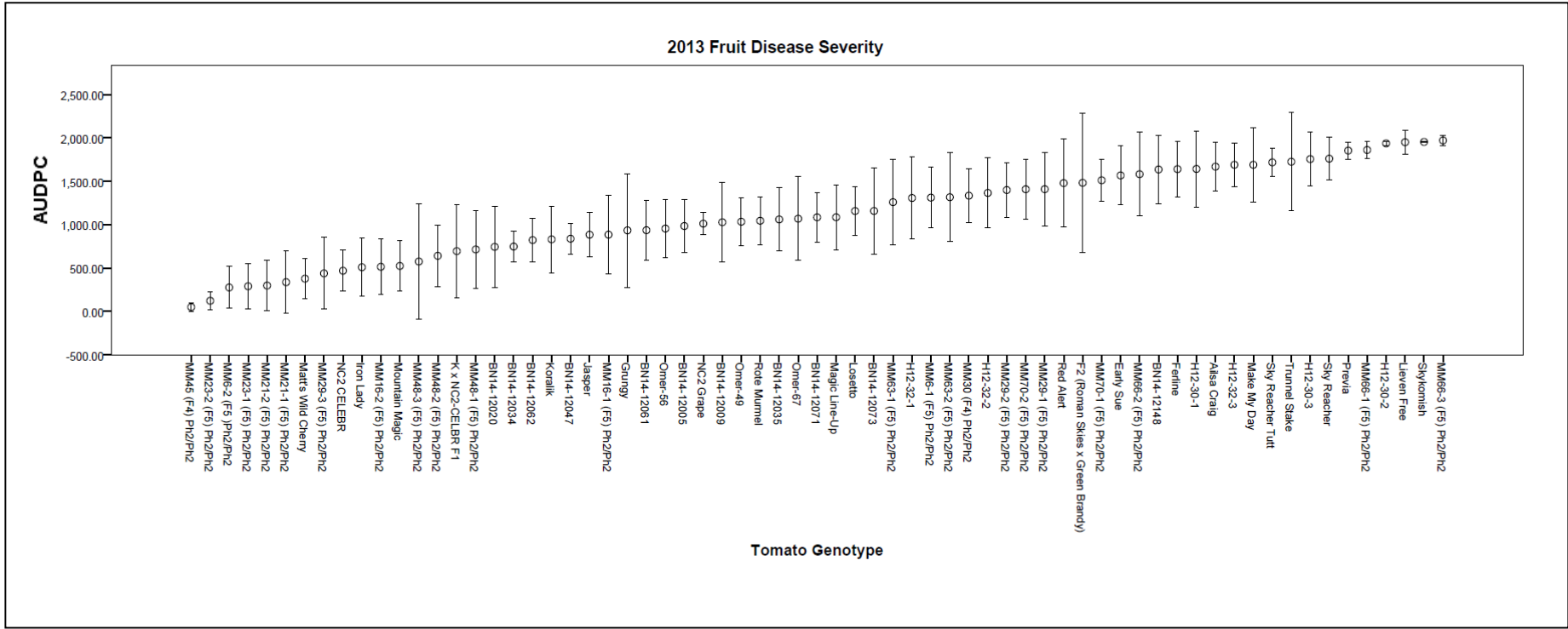
Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.





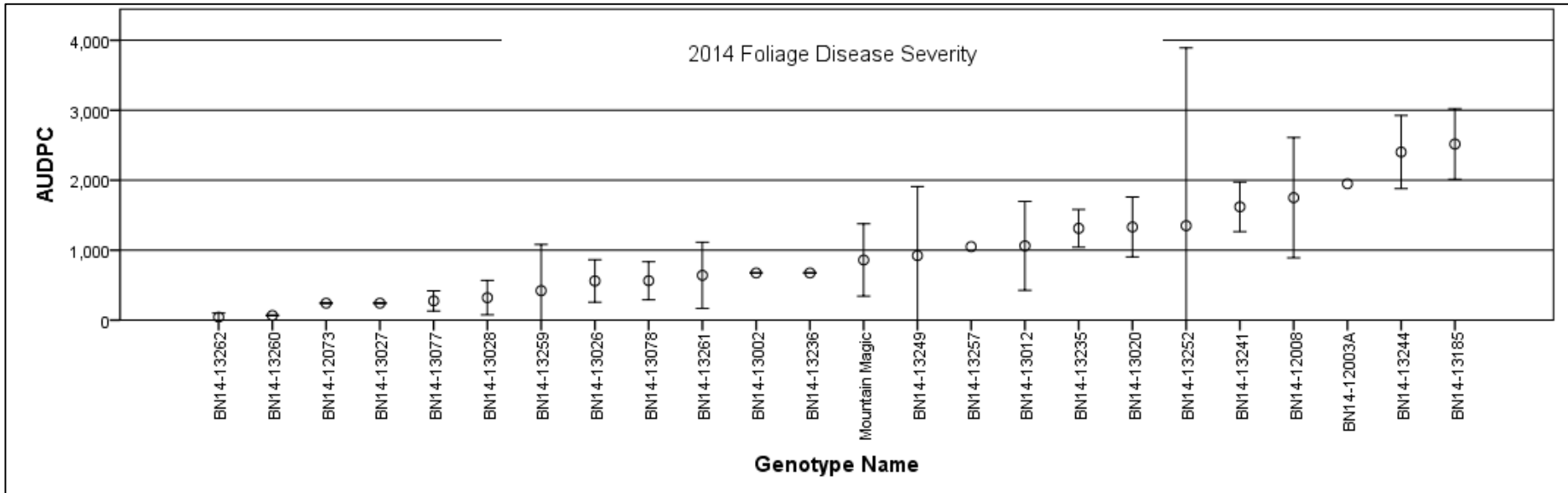
**Figure 2.10: 2013 foliage disease severity.**

Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.



**Figure 2.11: 2013 fruit disease severity.**

Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.



**Figure 2.12: 2014 Foliage disease severity.**

Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.

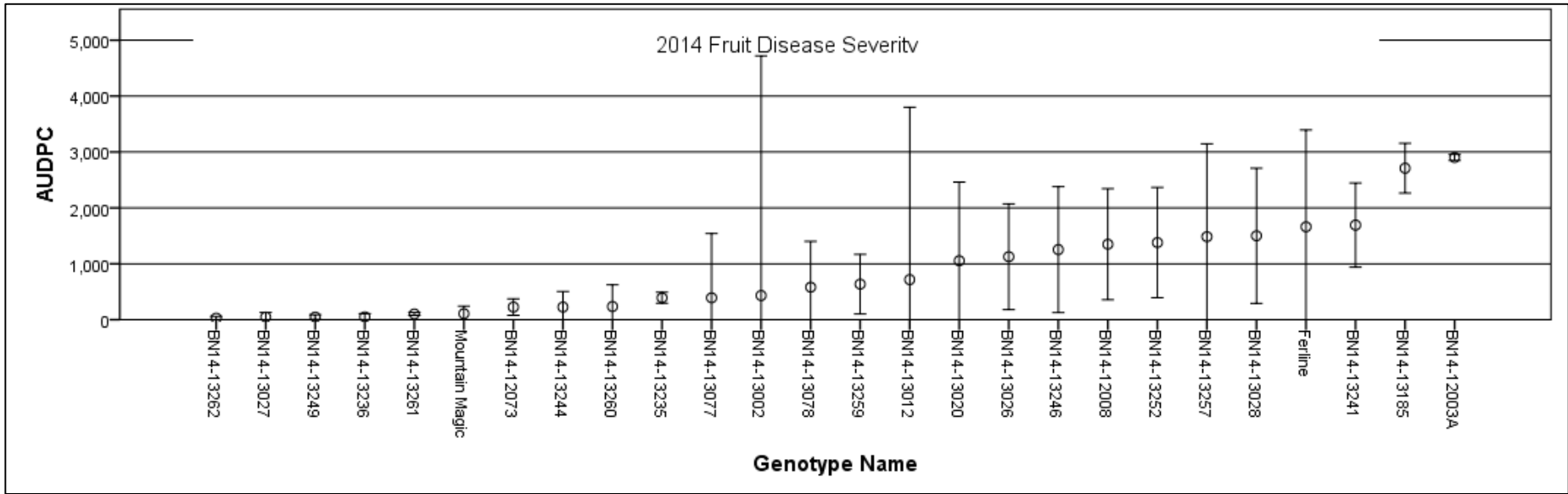


Figure 2.13: 2014 Fruit disease severity

Mean AUDPC for all tomato lines, arranged by increasing disease severity. Where present, error bars indicate 95% confidence interval.

### 2.3.7 Effect of *Ph*- genotype on disease severity

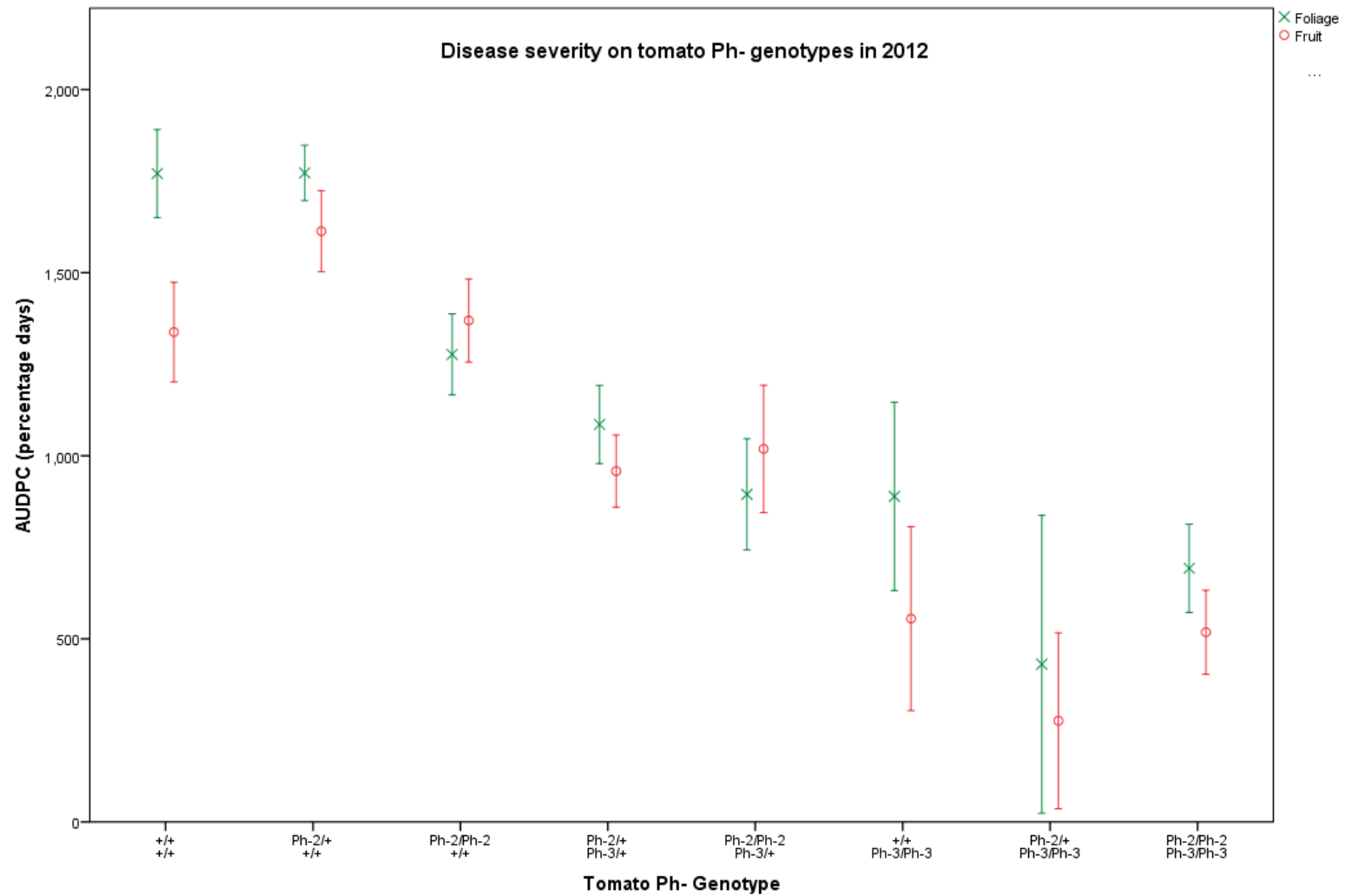
**Figure 2.14** and **Figure 2.15** show the mean resistance of each *Ph-2* and *Ph-3* (combined) genotype. Two-way ANOVAs considering *Ph-2* and *Ph-3* genotype as independent variables found significant effects of *Ph-2* and *Ph-3* genotype on both fruit and foliage disease severity (below). A significant interaction between *Ph-2* and *Ph-3* genotype for fruit disease severity was detected in the 2013 trial ( $p = 0.019$ ) although not in 2012 ( $p = 0.063$ ).

#### 2.3.7.1 Effect of *Ph*- genotype on foliage disease severity

Two-way ANOVA indicated that *Ph-2* genotype had a significant effect on foliage disease severity in both 2012 and 2013 ( $p < 0.001$  both years). *Post-hoc* tests showed significant differences between all homozygous null, heterozygous, and homozygous *Ph-2* genotypes in 2013 (in all comparisons,  $p < 0.001$ ), although in 2012 no significant difference was detected between lines heterozygous and homozygous for *Ph-2* ( $p = 0.693$ ). As with *Ph-2*, *Ph-3* was found to have a significant ( $p < 0.001$ ) effect on foliage disease severity in 2013, with all *post-hoc* comparisons significant at  $p < 0.001$ . In contrast, no significant overall effect of *Ph-3* genotype on foliage disease severity was detected in 2012. A significant interaction between *Ph-2* and *Ph-3* genotype was detected in 2013 ( $p = 0.001$ ) but not in 2012 ( $p = 0.068$ ).

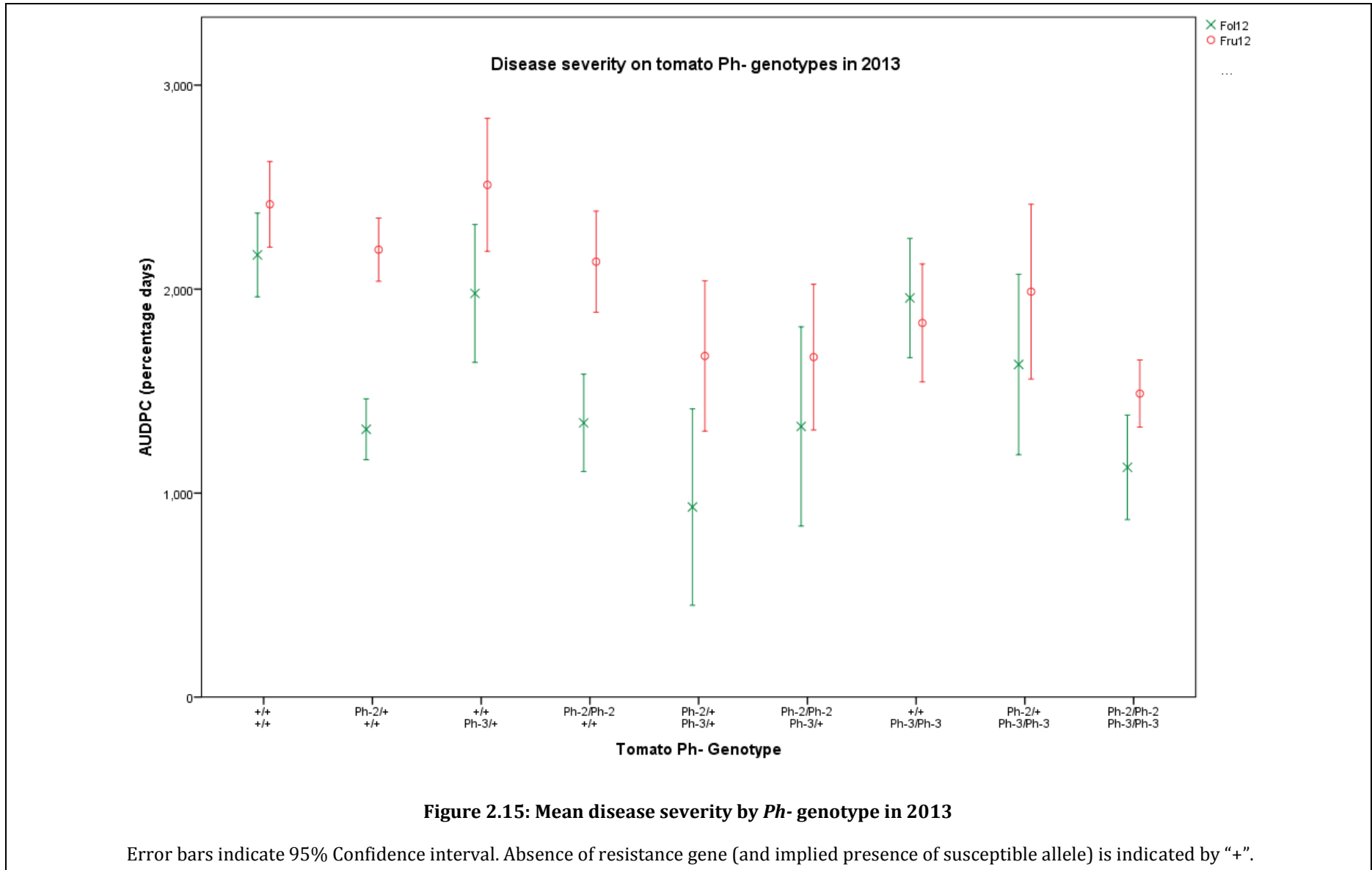
#### 2.3.7.2 Effect of *Ph*- genotype on fruit disease severity

Two-way ANOVA did not detect a significant effect of *Ph-2* genotype on fruit disease severity in 2013 ( $p = 0.965$ ), although the effect was significant in 2012 ( $p < 0.001$ ). *Post-hoc* tests showed that in 2012, homozygous *Ph-2* lines were significantly more resistant to fruit disease than either heterozygous lines ( $p = 0.036$ ) or homozygous susceptible lines ( $p = 0.001$ ). No significant difference was detected between lines with one copy of the *Ph-2* gene and homozygous susceptible lines ( $p = 0.390$ ). *Ph-3* had a significant effect on fruit disease severity ( $p < 0.001$ ) in both years. *Post-hoc* tests showed significant differences ( $p < 0.001$ ) between all *Ph-3* genotypes, in the 2013 trial. In 2012, no significant difference was detected between lines heterozygous and homozygous for *Ph-3* ( $p = 0.703$ ) although homozygous susceptible lines differed significantly from both heterozygous lines ( $p = 0.003$ ) and homozygous resistant lines ( $p < 0.001$ ).



**Figure 2.14: Mean disease severity by *Ph*- genotype in 2012**

Error bars indicate 95% Confidence interval. Absence of resistance gene (and implied presence of susceptible allele) is indicated by “+”.



### 2.3.8 Resistance in SL11- families

All 39 SL11- lines included in the 2011 field trial belonged to four families (**Table 2.9**). The mean foliage disease severity differed significantly between families ( $p < 0.001$ ). No significant effect of family on fruit disease severity was detected ( $p = 0.91$ ).

**Table 2.9: Fruit and foliage disease severity on SL11- families**

Mean AUDPCs of the four SL11- families in the 2011 polytunnel trial (fruit) and 2011 combined standardised polytunnel and field trials (foliage), and the results of a Kruskal-Wallis test of significant effect of family on disease severity. In each case, N = number of individual plants belonging to that family.

Family	Fruit		Foliage	
	Mean AUDPC	N	Mean SAUDPC	N
F5 Balcony Red x (F4 Red Alert x Sub Arctic Cherry)	603	12	1.21	41
F5 Balcony Red x Yellow Pygmy	586	15	1.74	48
F5 Jolly Elf x Tumbling Tom Red	582	22	1.16	45
F5 Balcony Red x Koralik	534	44	0.987	107
<b>P</b>	<b>0.91</b>		<b>&lt;0.001</b>	

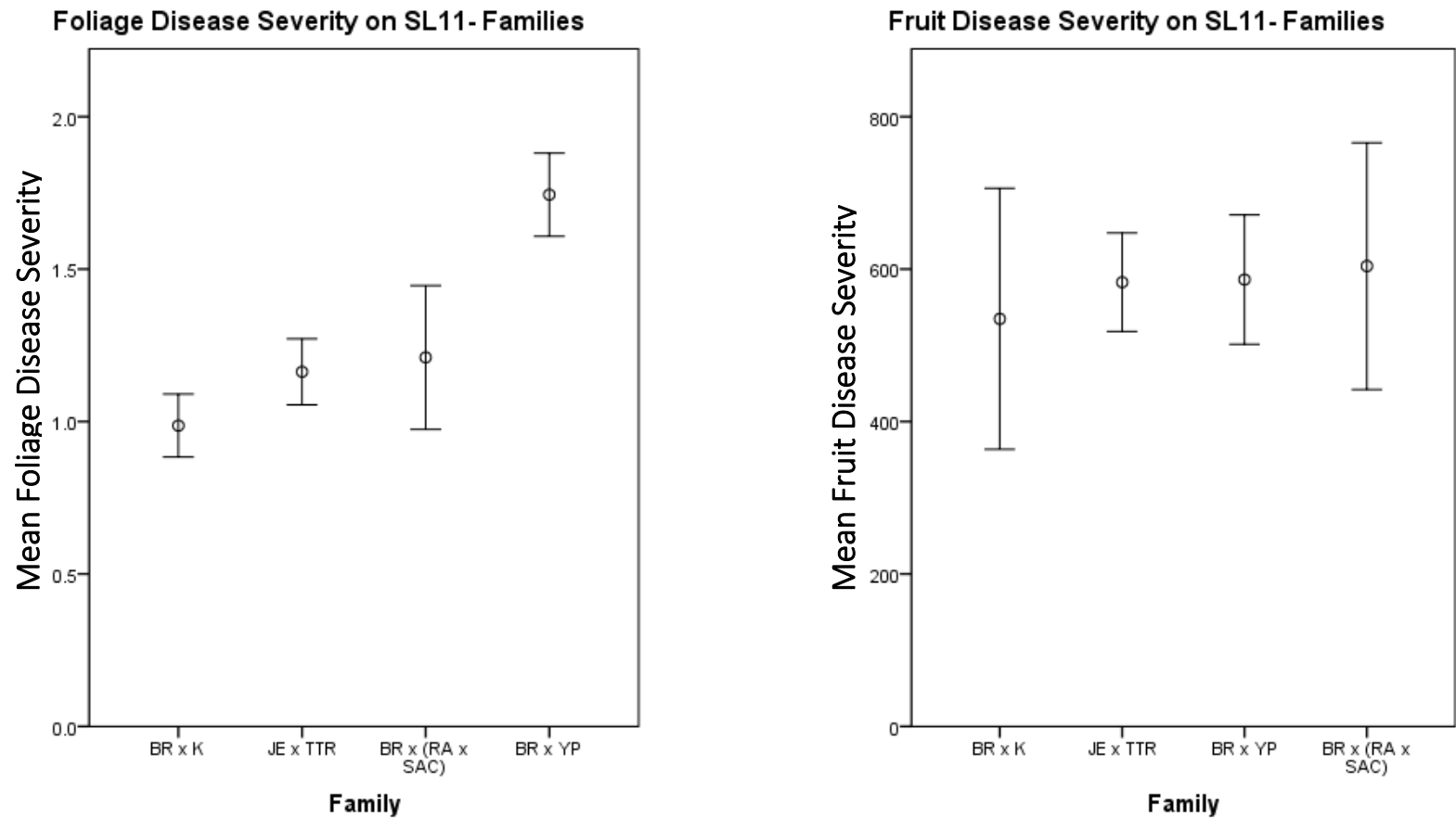
Post-hoc multiple comparisons (**Table 2.10**) confirm that, as suggested by the 95% CI error bars in **Figure 2.16**, Balcony Red x Yellow Pygmy lines were significantly more susceptible than the other families. Balcony Red x Koralik was suffered less foliage blight than any other line, although this difference was only significant with respect to Balcony Red x Yellow Pygmy.

**Table 2.10: Significant differences between SL11- Families**

Results of Dunn's Test (Bonferroni corrected) for significant difference of mean rank of foliage disease severity on SL11- families in the 2011 trial (combined polytunnel and field). Kruskal-Wallis chi-squared = 26.9101, df = 3, p-value <0.001

	F5 Balcony Red x (F4 Red Alert x Sub Arctic Cherry)	F5 Balcony Red x Koralik	F5 Balcony Red x Yellow Pygmy
F5 Balcony Red x Koralik	Z = -4.84 P < 0.001 **		
F5 Balcony Red x Yellow Pygmy	Z = -6.20 P < 0.001 **	Z = -1.49 P = 0.4101	
F5 Jolly Elf x Tumbling Tom Red	Z = -3.41 P = 0.002 *	Z = 2.32 P = 0.0609	Z = 4.01 P = 0.0002 **



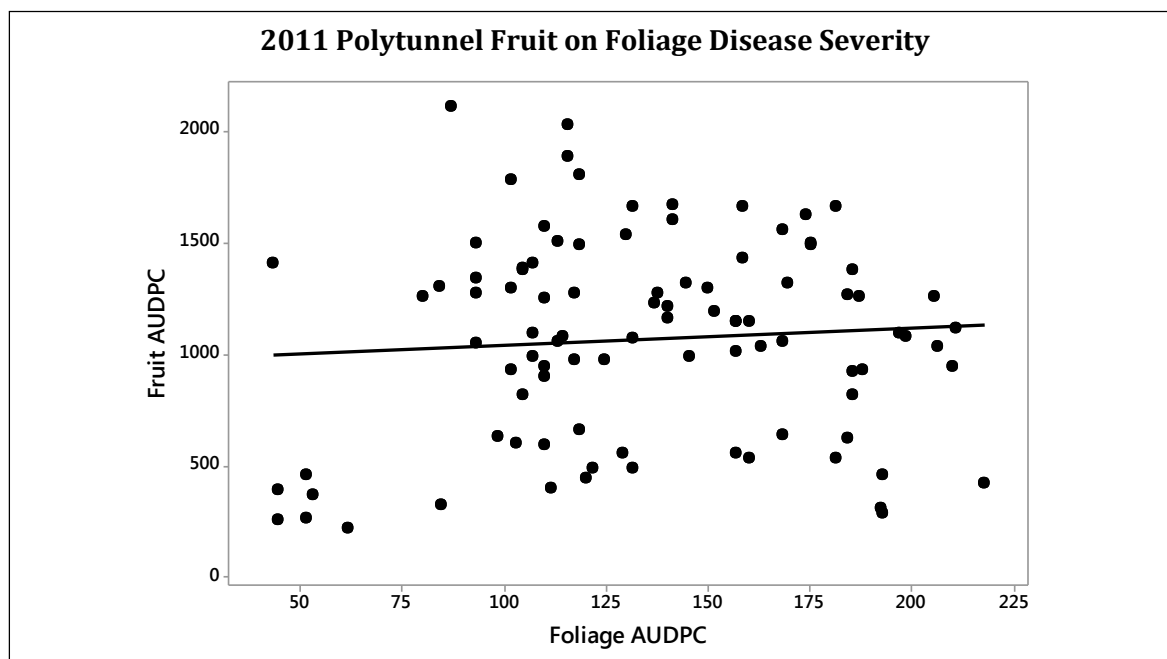


**Figure 2.16: Mean fruit and foliage disease severity on SL11- families**

Abbreviations: BR = Balcony Red, K = Koralik, JE = Jolly Elf, TTR = Tumbling Tom Red, RA = Red Alert, SAC = Sub-Arctic Cherry. Mean disease severity is in percentage days. Error bars indicate 95% confidence interval. There was no significant difference between families in mean fruit disease severity, although BR x YP lines were significantly more susceptible than the other families on average.

### 2.3.9 Correlation between foliage and fruit disease severity

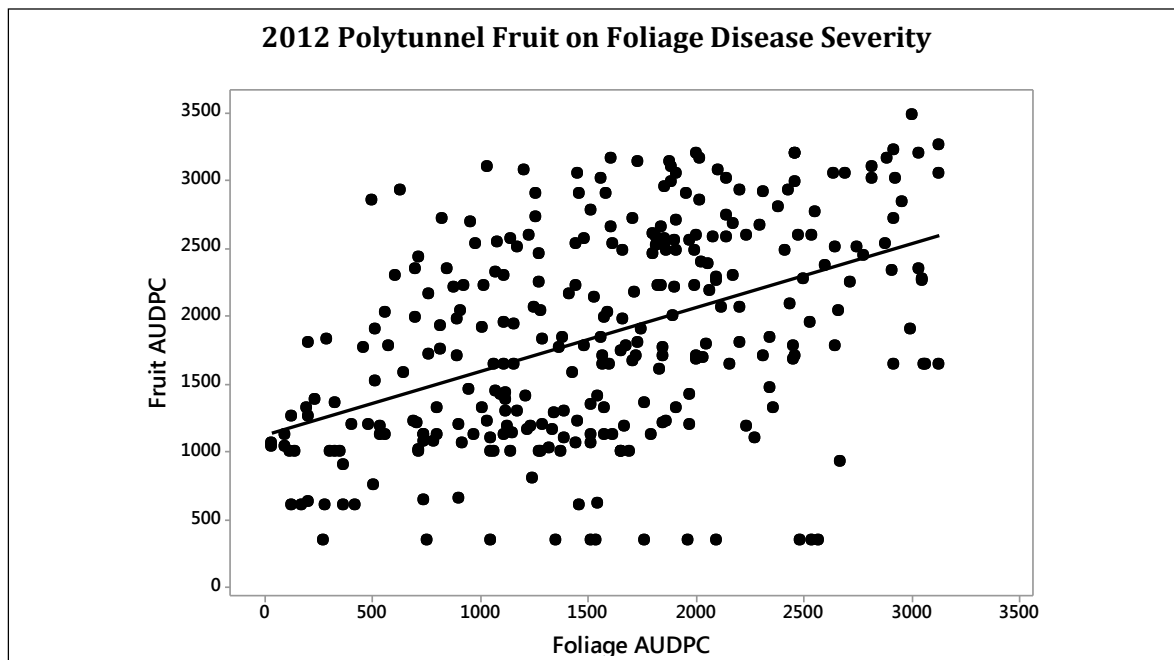
In the 2011 polytunnel trial, no significant effect of foliage disease severity on fruit disease severity was detected (**Figure 2.17**).



**Figure 2.17: Scatterplot of Fruit against Foliage AUDPC for 2011 tunnel trial.**

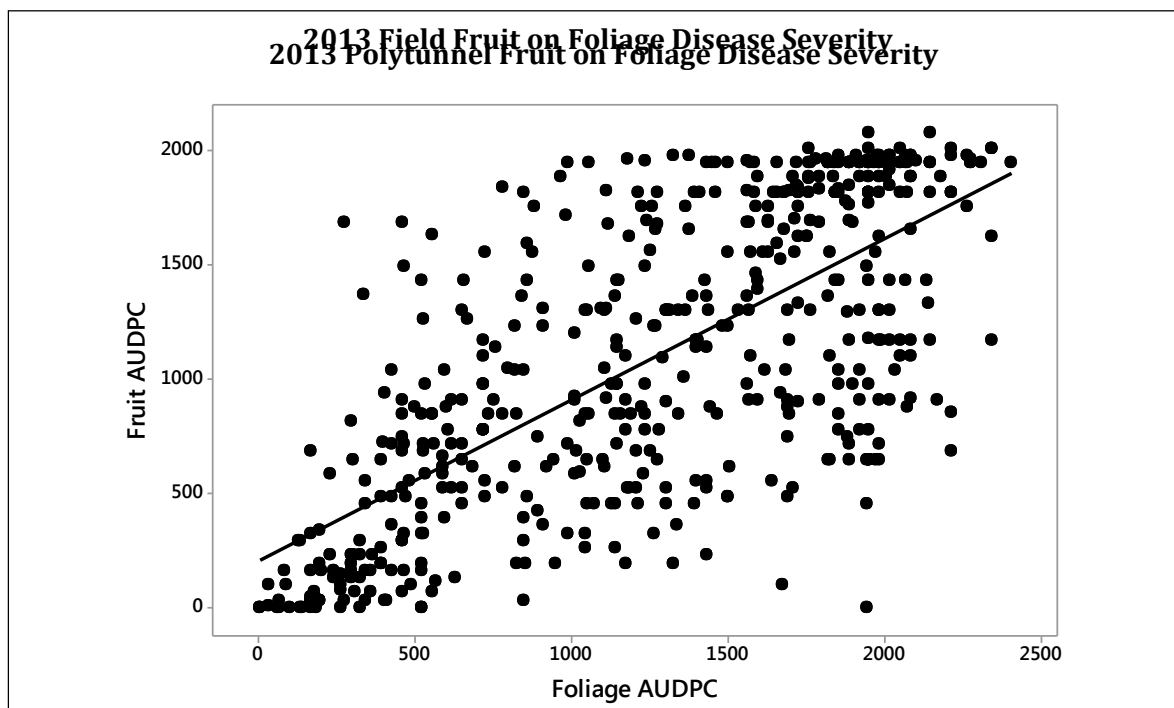
Fruit AUDPC plotted against Foliage AUDPC (percentage days) for individual plants in the 2011 polytunnel trial. Pearson correlation ( $r$ ) = 0.074,  $p$  = 0.476 (NS)

In 2012, a stronger (but still not significant) correlation was detected between disease severity on fruit and on foliage (**Figure 2.18**). However, in 2013, the correlation between fruit and foliage blight was obvious (**Figure 2.19**), strong (Pearson correlation = 0.701) and highly significant ( $p < 0.001$ ).



**Figure 2.18: Scatterplot of Fruit against Foliage AUDPC for 2012 trial.**

Fruit AUDPC plotted against Foliage AUDPC (percentage days) for individual plants in the 2012 field trial. Pearson correlation ( $r$ ) = 0.128,  $p$  = 0.221 (NS)

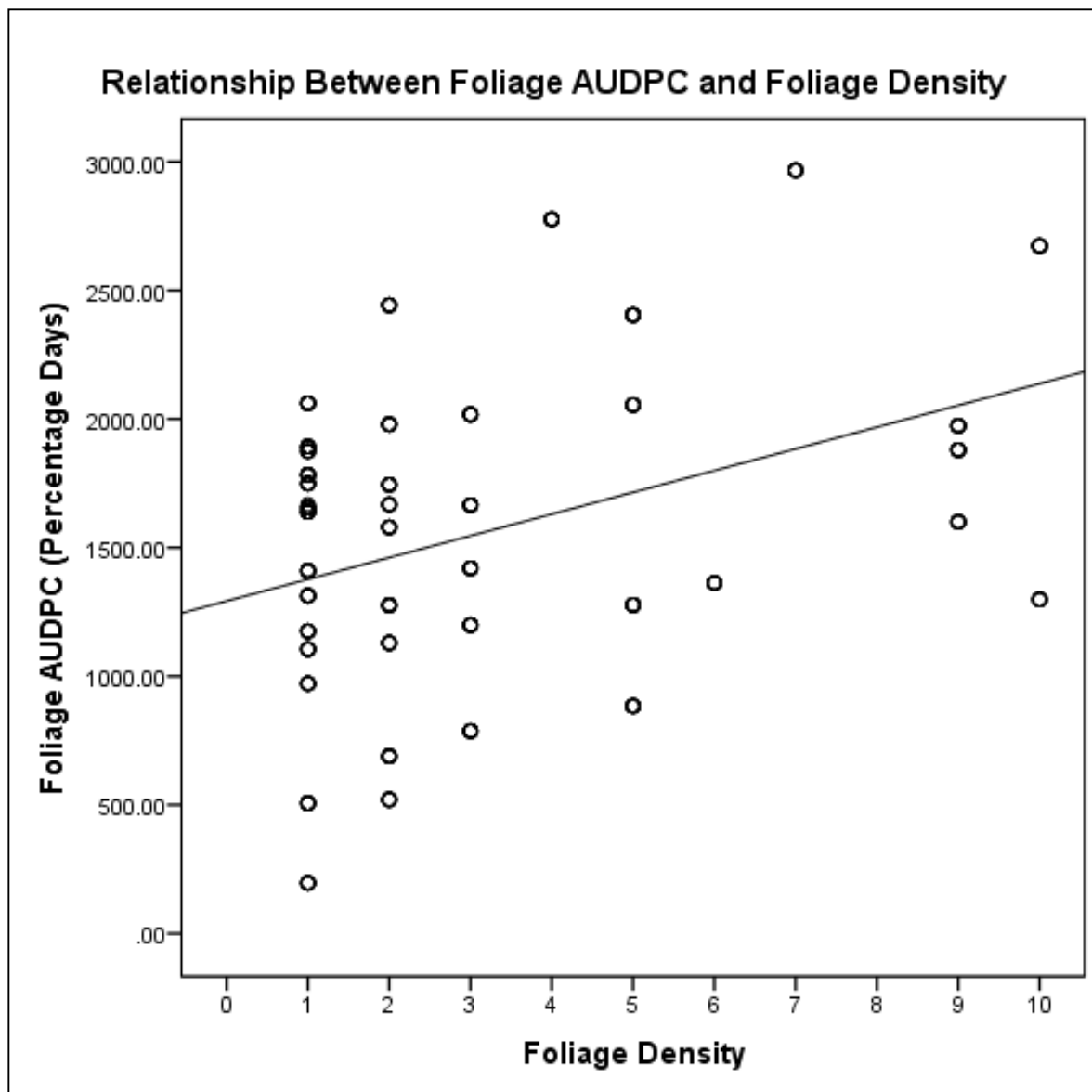


**Figure 2.19: Scatterplot of Fruit against Foliage AUDPC for 2013 trial.**

Fruit AUDPC plotted against Foliage AUDPC (percentage days) for individual plants in the 2013 field trial. Pearson correlation ( $r$ ) = 0.701,  $p$  < 0.001

### 2.3.10 Correlation between foliage density and disease severity

When the foliage disease severity was plotted against foliage density using the 2012 trial data (Figure 2.20), a weak (Pearson correlation = 0.270), but highly significant ( $p < 0.001$ ) correlation between increasing foliage density and increasing foliage disease severity was apparent.



**Figure 2.20: Scatterplot of foliage disease severity against foliage density**

Foliage disease severity as mean AUDPC (percentage days) for each tomato line in the 2012 field trial. Spearman's Rho = 0.270,  $p < 0.001$ . Foliage density is estimated on a rank scale where 1 = least dense and 10 = densest.

## 2.4 Discussion

### 2.4.1 Differentiation of *P. infestans* resistance level

In all years, there was a great degree of variation within lines, leading to wide 95% confidence intervals, particularly in 2011 and 2014, where line replication number was lower. However, it is nonetheless clear that there were genuine differences between lines, as lines at either tail of the distribution of *P. infestans* susceptibility-resistance were readily distinguishable (**Figure 2.5** to **Figure 2.13**). Disease severity on susceptible controls (Ailsa Craig and Red Alert) and lines shown to carry neither *Ph-2* nor *Ph-3* (for example, Silvery Fir Tree and Giant Syrian in 2012, and NC2 Grape and Sky Reacher Tutt in 2013) was high, and these lines produced little or no harvestable fruit (*ad lib* personal observations).

Disease pressure within the trial plot was certainly heterogeneous (particularly in 2012-2013) as the long, narrow trial plot in Harper's Field (**Figure 2.1** and **Figure 2.2**) was much more exposed, and therefore drier, at its Northern end than the Southern end, which was enclosed by hedges and buildings and tended to experience less air flow and direct sunlight. A more sophisticated analysis might have included the position of each plant in the field as a co-variate. The arrangement of replicate plants was stratified with respect to this gradient (along the long axis of the plot) so it is unlikely that the mean AUDPC of a given tomato line would be greatly altered relative to the other lines by this environmental gradient, but the variation in environmental conditions is likely to have contributed to the (often large) standard errors and 95% confidence intervals associated with the mean AUDPC values.

Undoubtedly, the 2011 and 2014 trials were less scientifically rigorous than those in 2012 and 2013, as both of the former lacked adequate replication or randomisation. In 2014 in particular, weather conditions and commercial considerations meant that the trial plot was inoculated earlier than in 2012 or 2013, it did not become heavily blighted until early October, by which time many plants were senescing for reasons other than *P. infestans* infection. However, the 2012 and 2013 trials can be considered reasonably robust assessments of the *P. infestans* resistance of a wide range of tomato cultivars.

### 2.4.2 Powdery mildew resistance

Powdery mildew infection on many lines was very heavy, particularly when tomatoes were grown (without *P. infestans* inoculation) in the glasshouse or polytunnel as part of the detached leaflet experiments described in **Chapter 4** and **5**, and as part of additional commercial demonstrations of Pro-Veg and Burpee lines grown in optimal conditions in a polytunnel in 2012

and 2014 (See **Appendix 8.3**). In these situations, powdery mildew infection was deemed by the authors to be the most serious ailment affecting the tomato plants. Whilst mildew was secondary to *P. infestans* and cold weather in terms of its effect on plants the field, it was the most important pathogen after *P. infestans*. Resistance to powdery mildew is conferred by a large number of genes, some of which are recessive (Kole 2007) so molecular marker screening for these genes was not attempted. However, the field performance recorded here (**Table 2.7**) was used by Burpee in selecting lines for their breeding programme (Simon Crawford, Burpee Europe Ltd., *pers. comm.*).

### 2.4.3 Relationship between foliage and fruit disease severity

No significant correlation between fruit and foliage late blight disease severity was observed in 2011 or 2012, although a highly significant correlation was in detected 2013 (**Figure 2.17** to **Figure 2.19**). Foolad *et al.* (2008) found that *S. pimpinellifolium* accessions with resistant foliage suffered high levels of fruit infection, demonstrating that resistance in fruit and foliage may be independent. However, some level of correlation in the field was expected, as heavily infected foliage is likely to shed sporangia onto fruit at a high rate, leading to more frequent fruit infections. Butz (2010) found a correlation between foliage and fruit infection level in the field, although not in laboratory tests of the same plant material, which seems to support this explanation.

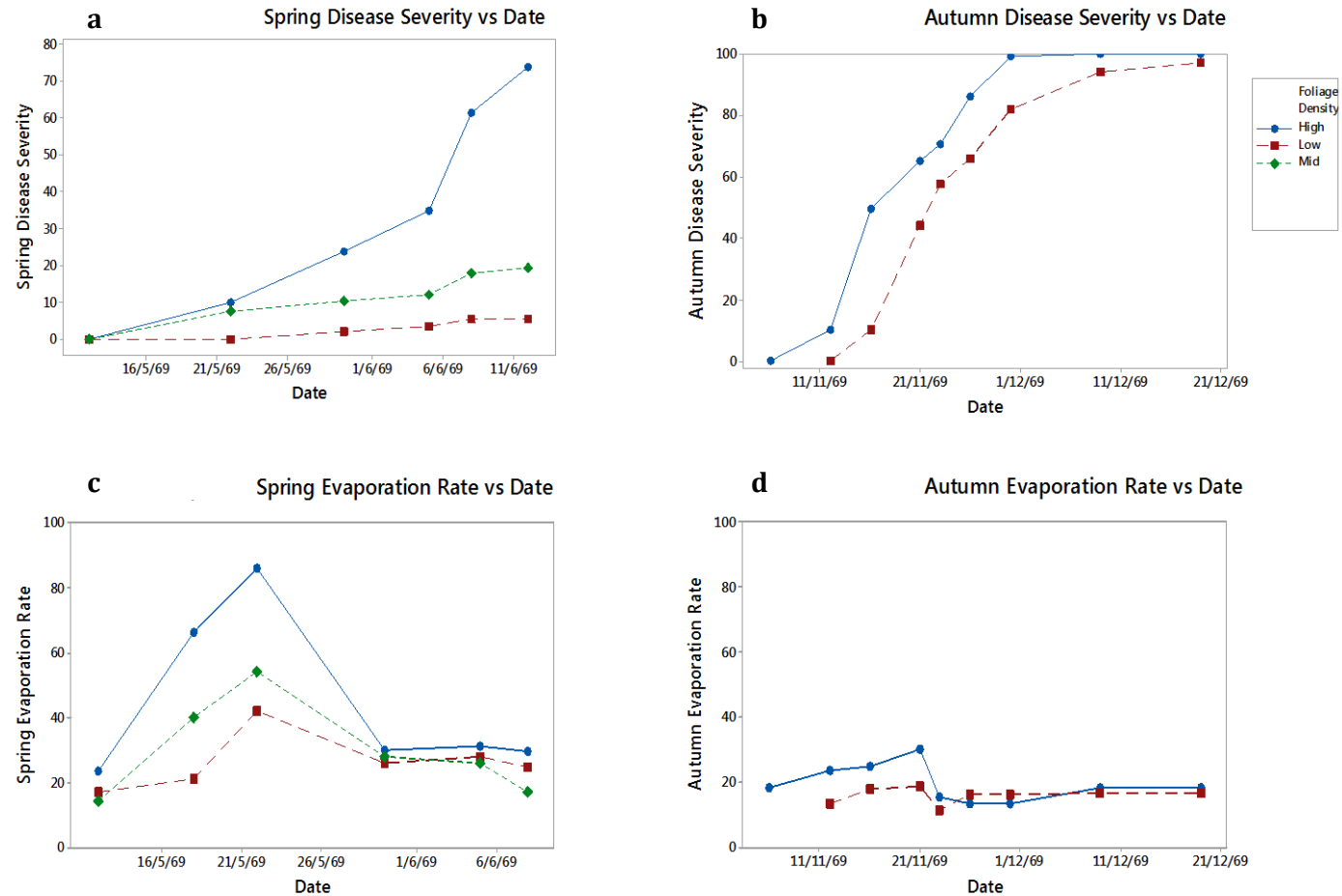
### 2.4.4 Comparison of SL11- Families

The SL11- lines containing Koralik as a parent were the least susceptible to fruit and foliage disease (

**Table 2.9**), suggesting Koralik was the source of the *P. infestans* resistance. However, in neither the case of fruit nor the foliage blight was the mean disease severity on the Koralik crosses significantly lower than that on other lines. Koralik had previously been selected as a parent in these crosses by John Burrows of Pro-Veg Seeds Ltd. (John Burrows, Pro-Veg Seeds Ltd., *pers. comm.*) as it is anecdotally reported to have good blight resistance. Whilst no scientific literature discussing this could be found, Peirce (2009) reported that Koralik performed well in an informal garden trial, and it had likewise performed well in previous informal trials conducted by Pro-Veg (John Burrows, Pro-Veg Seeds Ltd., *pers. comm.*), and in the present 2013 field trial. The disease severity on Koralik-derived lines ranged from well above to well below the other lines in the 2011 trial, and was not significantly different from any family apart from the highly susceptible Balcony Red x Yellow Pygmy family. Therefore, the results of this trial do not prove or disprove Koralik has exceptional resistance.

#### 2.4.5 Foliage Density

There was a clear correlation between foliage blight resistance and foliage density, with less dense foliage tending to exhibit less foliage blight (**Figure 2.20**), probably owing to the lower humidity levels within the foliage and reduced tendency to trap liquid water. Rotem and Ben-Joseph (1970) conducted experiments measuring the rate of foliage disease progression in plots of potato plants with different foliage density, and found that in the autumn, when weather conditions were optimal for *P. infestans* growth, the planting density had little effect on the rate of disease development (**Figure 2.21-b**) or on the rate of water evaporation within the canopy (**Figure 2.21-d**). However, in the spring, when weather conditions were warmer and drier and therefore more marginal for *P. infestans* growth, disease severity increased markedly more quickly in the more densely planted plots (**Figure 2.21-a**) and the rate of water evaporation was markedly higher in less dense plots (**Figure 2.21-c**). The situation in field crop of potatoes is somewhat different to that in the trials conducted in the present study, insofar as the former consists of a large area of more or less continuous crop canopy, whereas in the latter case, plants were isolated and discrete. However, there would not appear to be any reason why the broadly same relationship between foliage density and rate of disease progress (presumably due to the effect of foliage density on the local microclimate) should not apply. In 2012, when the present measurements were made, conditions were exceptionally wet (Met Office 2015) and therefore favourable for *P. infestans* growth and spread, so in a drier summer the strength of the correlation detected may have been even greater.



**Figure 2.21: Influence of foliage density on *P. infestans* progression.**

From (Rotem and Ben-Joseph 1970). Top graphs show the progress of foliage blight on potato planted at different densities in summer (a) and winter (b). Bottom graphs show evaporation rate measured with a Piche evaporimeter in the potato foliage. Density has a strong effect on evaporation and *P. infestans* disease progression during warm, dry spring conditions (c) but little effect during autumn conditions optimal for *P. infestans* growth (d).



#### 2.4.6 *Ph-2* and *Ph-3* marker genotypes

Tomato lines with both the *Ph-2* and *Ph-3* resistance genes (for example, Mountain Magic, 12005-12073, Magic Line Up, several MM F5 lines (#29 - #44 and #56 - #62), NC2 CELBR and Koralik x NC2-CELBR in 2013, and Mexico Midget, Rote Murmel and Defiant in 2012, showed very high levels of resistance, whereas lines with only one of the two genes generally showed much higher levels of disease severity, in line with reports by other workers (Brusca 2003; Kim and Mutschler 2006; Nowicki *et al.* 2013; Ozores-Hampton and Roberts 2014; Wagner 2012; Zhang *et al.* 2013).

Genotyping using the TG328 *Ph-3* marker was technically simple, unlike the *Ph-2* markers, which amplified weakly and produced similar sized fragments for the R and S allele, making resolution of the fragments on a gel difficult. However, TG328 (and other *Ph-3* markers) produced anomalous results. Some lines which were known to be susceptible and not to contain the *Ph-3* gene(s), based on published genotype information and supported by their phenotype in the field trials – (e.g. NC2-Grape (Gardner 2008), **Table 2.7**) produced a resistant allele fragment. This was the case when the tests were repeated multiple times with batches of DNA extracted from different individual plants (data not shown). This situation would seem to suggest that “resistant” marker alleles not linked to functional *Ph-3* genes exist. The fact that different variants of the TG328 marker (i.e., TG328-F2/R2 digested with either *Bst*NI or *Apo*I) sometimes gave conflicting results (for example, Mountain Magic, Previa, and Ferline, **Table 2.7**) adds further confusion, although the authors suggest that results obtained by digesting the marker with *Apo*I are likely to be more accurate (Foolad and Panthee 2013). Ultimately, markers may be population specific, and whilst the markers used here have been demonstrated to give consistent results in a few tomato lines (Foolad and Panthee 2012), this does not guarantee that they will give consistent results across the wide range of tomato germplasm tested in the present study. A number of alternative *Ph-3* markers have been developed in recent years (some as a by-product of efforts to fine-map *Ph-3*). Several alternative *Ph-3* markers are listed in **Table 2.11**. Unfortunately, it was not possible to trial all of these markers in the current project, but a side-by-side comparison of these markers against a range of known tomato germplasm would be a useful future study. The SCAR marker described by Truong *et al.* (2013) appears especially useful, as it is co-dominant, produces well separated fragments, and requires only PCR and no enzymatic digestion step, and gave the expected results in a wide range of tomato germplasm.

**Table 2.11: Alternative *Ph-3* resistance gene markers**

Name	Type	Description	Study
TOM236	SSR	BSA was conducted, and 5 susceptible and 5 resistant F <sub>2</sub> individuals plus the parents were genotyped. TOM236 produced a 185 bp fragment when tested with the susceptible lines and a 155 bp fragment when tested with the resistant lines.	Zhu <i>et al.</i> (2006)
CCPB272-03	RAPD	BSA indicated that <i>Ph-3</i> was within 5.8 cM of CCPB272-03.	YiPeng <i>et al.</i> (2009)
<i>Ph-3-L</i> and <i>Ph-3-R</i>	SCAR	A dominant SCAR marker was designed from previously identified AFLP markers linked to <i>Ph-3</i> in L3708 (Chunwongse <i>et al.</i> 2002) and tested against 19 inbred lines of known <i>Ph-3</i> genotype. A 123 bp fragment was amplified in the case of resistant lines.	Park <i>et al.</i> (2010)
Indel_3 and P55	CAPS (Indel_3) and Dominant (P55)	A cross was made between L3708-derived resistant line CLN2037B and a susceptible line. Markers Indel_3 and P55 were found to associate with a resistant phenotype and flank a 74 Kb region containing four Resistance Gene Analogues.	Zhang <i>et al.</i> (2013)
SCU602F3R3	SCAR	This marker produced a 400 bp fragment in the case of resistant lines 450 bp fragment in the case of susceptible lines. Tested against a wide range of lines and was consistently in agreement with phenotype and/or published genotype and/or Park <i>et al.</i> (2010).	Truong <i>et al.</i> (2013)
Unnamed	SCAR	In a series of crosses between NC2 CELBR and 33 susceptible parents, 7 indels were identified 0.05 to 0.21 Mb from <i>Ph-3</i> , and SCAR markers (not published) were developed from these.	Shekasteband <i>et al.</i> (2014)

## 2.5 Conclusions and Recommendations

Mountain Magic, Defiant, Rote Murrel, Matt's Wild Cherry, and several of the *S. pimpinellifolium* accessions appeared to stand well above most other lines in terms of foliage and fruit blight resistance, and overall plant health. Additionally, a number of "MM F5" lines, in development by Burpee, showed very high foliage and fruit blight resistance in the 2013 trial. Correlation between fruit and foliage blight was weak. Plant architecture appears to have an important effect on blight tolerance, clearly favouring cordons with sparse foliage over dense bush types. As is well documented, *S. pimpinellifolium*-derived cultivars exhibit good blight tolerance. Direct inoculation of trial plants appears to be a more reliable way to ensure infection than use of spreaders and wind-blown/rain splashed spores. Whilst Hazelnoot Tomaat and Mexico Midget produced few fruit in this trial, they would appear to be worthy of further consideration in future trials, and it would be interesting to see how they performed with earlier planting and more favourable conditions.

Most of the germplasm trialled here relied on *Ph-2* and/or *Ph-3* for its resistance, with possible involvement of other genes in some of the "currant tomato" material. However, an active

programme is underway at Pennsylvania State University to transfer *Ph-5.1* and *Ph-5.2* resistance from *S. pimpinellifolium* to elite tomato backgrounds (Nowicki *et al.* 2013). Additionally, a resistance gene named *Ph-4* was identified in *S. pimpinellifolium* accession L3708 (the source of *Ph-3*) by Kole *et al.* (2006) but no breeding programmes appear to be attempting to use it at present. Transfer of minor gene resistance to cultivated tomatoes is discussed in **Chapters 1 and 5**, although it is worth noting here the performance of the line “Matt’s Wild Cherry”, which proved highly resistant to fruit and foliage disease in the present study. Hansen *et al.* (2014), Johnson *et al.* (2014) and López Kleine *et al.* (2012) reported that the resistance of Matt’s Wild Cherry is only partially overcome by the most aggressive *P. infestans* isolates, and that the source of the resistance is currently unknown by the phenotypic response suggests that it is distinct from *Ph-2* and *Ph-3*. McGrath (2013) stated that the resistance was “possibly” due to *Ph-3* – a finding in line with the ambiguous *Ph-3* genotype detected in the present study. Whilst the resistance in Matt’s Wild Cherry has clearly come to the attention of these research groups, at present no work has been published regarding mapping the source of the resistance or transferring it to novel cultivars, and these would clearly be useful objectives for future research.

Finally, in January 2015 a novel F<sub>1</sub> hybrid tomato cultivar named “Crimson Crush” was registered and released on the UK market by Burpee (being sold under license by Suttons Seeds Ltd. in 2015). Crimson Crush is a direct product of the breeding project of which these trials were a part, and its field resistance and *Ph*- genotype were verified in these trials. The details of its pedigree are not publicly available at present, for reasons of commercial confidentiality.

## 3 An investigation into *Phytophthora infestans* diversity and host specialisation in British gardens and allotments

### 3.1 Introduction

#### 3.1.1 Impact of *P. infestans* on potato and tomato crops

*Phytophthora infestans* is the causal agent of late-blight disease, one of the most serious diseases of potato (*Solanum tuberosum*) and outdoor tomato (*S. lycopersicum*) crops worldwide. On potato, *P. infestans* causes yield reductions due to defoliation, increased production costs associated with fungicide sprays, and further losses of tubers in the field and in storage (Fry 2008b). In infected tomato crops, yield reduction may be caused by the rapid destruction of foliage, but this mechanism is relatively unimportant compared to infection of the fruit itself, which occurs readily in unsprayed organic crops of most cultivars (Nowicki *et al.* 2012). Infected fruits develop firm brown lesions within 4-16 days, which begin to sporulate within 5-16 days (Butz 2010). Infected fruits also often develop secondary bacterial or fungal infections. Symptoms on foliage may develop much more quickly, however, and under favourable conditions, *P. infestans* can cause 100% tomato crop loss within 7-10 days (Fry 2008b; Nowicki *et al.* 2012; Sharma *et al.* 2008).

#### 3.1.2 Recent and historical *P. infestans* migrations

Worldwide migrations of *P. infestans* are discussed in detail in **Chapter 1**. The geographical origin of *P. infestans* has historically been the subject of debate, with Gomez-Alpizar *et al.* (2007) presenting evidence for *P. infestans* having originated in the South American Andes. However, recent work by Goss *et al.* (2014) claims to have conclusively demonstrated the Mexican origin of the pathogen. Irrespective of the original centre of origin, Mexico and the South American Andes are today major centres of *P. infestans* diversity (Goss *et al.* 2014). The first spread of *P. infestans* from Mexico and South America took place in the 1840s, leading to the Irish and Scottish “Potato Famines” (Bourke 1964). These were caused by a single *P. infestans* lineage “HERB-1” of A1 mating type, which was subsequently displaced by the closely related US-1 lineage (Yoshida *et al.* 2013). In the late 1970s, a second migration of new genotypes (including those with A2 mating types) from Mexico to Europe and to USA and subsequently to the rest of the world (Hohl and Iselin 1984; Spielman *et al.* 1991) paved the way for matings, sexual recombination, and the rapid appearance of novel genotypes. Thus, since the early 1980s, *P. infestans* populations in most European countries have consisted of multiple unique genotypes and recurrent clonal lineages (Euroblight 2014).

### 3.1.3 Monitoring of *P. infestans* in Britain and Europe

*P. infestans* populations in Britain have been closely monitored on commercial potato crops since the mid-1990s (Cooke *et al.* 2007; Cooke *et al.* 2014; Cooke *et al.* 2003; Day *et al.* 2004). This work has shown that since 2007-2008, two strains, 13\_A2 and 6\_A1, have become dominant in most areas of Britain, with relatively few other clonal lineages present (Cooke *et al.* 2014). In Britain, there is little or no commercial outdoor tomato cultivation, and the glasshouse tomato crop is rarely blighted due to the warmer, dryer conditions in glasshouses, which do not favour *P. infestans* development (Collins 2013; Nelson 2008). However, amateur gardeners commonly grow outdoor tomato crops and experience late-blight outbreaks on these as well as on potato. In Britain most monitoring of *P. infestans* populations has taken place in commercial potato crops, and little is known about the *P. infestans* population on tomato, or the diversity of *P. infestans* present in domestic gardens and allotments (henceforth “gardens”). However, with over 150,000 allotment plots in England (Campbell and Campbell 2013), and outdoor tomatoes widely grown by amateur gardeners throughout Britain, there is potentially additional *P. infestans* diversity that has not yet been sampled.

On a European scale, *P. infestans* monitoring is co-ordinated by Euroblight ([www.euroblight.net](http://www.euroblight.net)), formerly EUCABLIGHT and EU.NET.ICP. Euroblight is an organisation of scientists and other professionals working primarily on potato late blight, with a recent additional focus on early blight caused by *Alternaria* species. Euroblight organises 18-monthly workshop meetings, curates *P. infestans* datasets, and facilitates international research collaboration.

### 3.1.4 Tools for monitoring *P. infestans* populations

In many European countries, including Britain, *P. infestans* spread is mostly by sporangia containing asexually produced zoospores, rather than by sexually recombinant oospores (Collins 2013; Cooke *et al.* 2014; Lees *et al.* 2012). Accordingly, the majority of *P. infestans* outbreaks are caused by isolates belonging to recurrent clonal lineages. Genetic variation originating from mutations does exist within these clonal lineages (Cooke *et al.* 2014), and novel clonal lineages do arise periodically through more extensive mutation, mitotic recombination, sexual recombination, or migration from *P. infestans* diversity hotspots such as Mexico (Goodwin *et al.* 1994a). For example, the clonal lineage 13\_A2 was first detected in the Netherlands and Germany in 2004, and in Britain in 2005 (Cooke *et al.* 2012; Shaw *et al.* 2007), and lineage 6\_A1 was first detected in the Netherlands in 2002 and in Britain in 2004 (Kildea *et al.* 2013). Several methods have been developed to classify *P. infestans* isolates into distinct lineages, and to establish how these lineages are related. These methods are discussed below.

### 3.1.4.1 Phenotypic characters

Prior to the advent of PCR and easy genotyping, *P. infestans* isolates were first characterized using phenotypic characters such as those discussed below. Despite the great advances in molecular taxonomy that have been made since the advent of PCR in the 1980s, phenotypic characters remain important in the taxonomy of *P. infestans* (and other organisms), and indeed, those discussed below are all of practical relevance to plant pathologists and agronomists in their own right.

- The mating type of a *P. infestans* isolate can be determined experimentally by inoculating it onto a plate of growth medium along with A1 and A2 reference isolates either side (Tantius *et al.* 1986). The colonies are allowed to grow until they make contact and the intersections between them are examined microscopically after 10-14 days (Bakonyi and Cooke 2004). Presence of oospores at the intersection with either isolate indicates that the isolate being tested is of the opposite mating type, and possible presence of a visible repulsion zone at the intersection with the other (David Shaw, Sarvari Research Trust, *pers. comm.*), indicates that the isolates are of these same mating type. The method is not completely reliable, as some isolates are self-fertile and able to form oospores without mating with another isolate (Tantius *et al.* 1986).
- Virulence testing is used to classify *P. infestans* isolates into physiological races. Race was an important taxonomic character prior to the advent of molecular markers, and remains so today on account of its obvious relevance to plant breeders. *P. infestans* isolates are tested against a set of 11 potato differential cultivars, (Black *et al.* 1953; Malcolmson and Black 1966; Malcolmson 1969) plus a susceptible clone such as Bintje or Craigs Royal. The differential cultivars were originally believed to carry single R genes, although at least one of these (the R3 differential) has now been shown to carry two closely linked genes with different specificities (Huang *et al.* 2004). A standardised race testing protocol has been developed by EUCABLIGHT members (Andrison *et al.* 2011). The *P. infestans* isolate under consideration is inoculated onto detached leaflets of the 12 differential clones. Development of a sporulating lesion after seven days is considered to indicate that the isolate overcomes the R gene carried by the inoculated leaflet. *P. infestans* races are named by listing the R genes against which they are virulent (e.g. *P. infestans* race 1,2,4 is capable of producing sporulating lesions on potato clones carrying R genes 1, 2, or 4, or any combination thereof, but not clones carrying any other R genes). A similar classification system based on tomato *Ph-* genes has been used to classify isolates by workers in Taiwan (Chen *et al.* 2014). No formal system has been proposed, but a number of workers have also inoculated isolates onto susceptible tomato and potato leaflets, to

test their aggressiveness in terms of lesion growth rate, on the two hosts. For examples of such studies, see **Table 1.1**.

- Fungicide sensitivity is another phenotypic trait that has obvious practical relevance in addition to its use in classifying isolates, and may be determined in vitro or in vivo (Shattock *et al.* 1990). For in-vitro testing, the isolate to be tested is inoculated onto plates of growth medium with and without fungicide added to the medium. Colony diameter after a period of time (typically 6-10 days) on the two plates is compared, and the isolate's sensitivity to the fungicide is typically classified onto a three-level scale as being sensitive, intermediate, or resistant when compared with standard isolates (Deahl *et al.* 1995).

#### 3.1.4.2 Early molecular techniques

Non-PCR-based allozyme and RFLP analysis have been widely used (**Table 1.1**). However, they require relatively large quantities of sample material to be grown and extracted, and the RG57 technique in particular is rather laborious and requires the use of radioactive probes, so both are arguably being superseded by PCR-based techniques. Contemporary studies do nevertheless make occasional use of these techniques (for example, Chowdappa *et al.* (2013)).

- Allozyme analysis by starch gel electrophoresis of *Gpi-1* (glucosephosphate isomerase) and *Pep* (peptidase) enzymes extracted from *P. infestans* isolates was first employed by Tooley *et al.* (1985). Variants of *Gpi-1* and *Pep* which migrate through starch gels at different rates exist, and can be used to distinguish *P. infestans* isolates.
- RFLP fingerprinting using the RG57 probe is performed by extracting DNA, digesting with *EcoRI* to yield DNA fragments (which are polymorphic between differing *P. infestans* isolates), electrophoresing, Southern blotting to a nylon membrane, and hybridising with the radio-labelled RG57 *Phytophthora* DNA probe to allow photographic visualisation of the bands each representing a different sized restriction fragment (Goodwin *et al.* 1992).

#### 3.1.4.3 PCR-based molecular techniques

As with many other fields of biological study, the taxonomy of Oomycetes and other similarly amorphous organisms has been revolutionised by modern PCR methods (Cooke and Lees 2004). Those routinely employed in the study of *P. infestans* are described below.

- AFLP Analysis (Vos *et al.* 1995) was first applied to *P. infestans* by Van der Lee *et al.* (1997). The technique consists of using restriction enzymes *EcoRI* and *MseI* to cut *P. infestans* template DNA at restriction sites. Adaptors (short lengths of DNA) designed to match the cut ends of the restriction sites, are included in the digestion mix, and ligate to the cut sites. PCR primers designed to anneal to the adaptors are then added, and PCR

amplification is performed, producing products of a length equal to the cut restriction fragments. These PCR products can be electrophoresed and visualised using either radio-labelled primers included at PCR setup, or alternatively using non-radioactive DNA stains. The “DNA fingerprints” thus produced can be compared and bands scored as present or absent. A great advantage of the AFLP technique is that it can be applied to taxa without any prior knowledge of their DNA sequence and produce reasonably consistent results (Jones *et al.* 1997). However, Jones *et al.* (1997) also reported some difficulty for new users of the technique in optimising their protocol and achieving consistent results, and in their study the results of AFLP were never as consistently replicated between laboratories as were those from SSR markers (below).

- Mitochondrial DNA Haplotype analysis makes use of four PCR primer pairs to amplify regions of *P. infestans* mtDNA (Griffith and Shaw 1998). The PCR products are then digested with *MspI* and *EcoRI* restriction enzymes to produce fragments which are polymorphic in length between differing *P. infestans* isolates. The fragments are separated electrophoretically and viewed with DNA stains. The method appears to be an implementation of the CAPS marker technique (Konieczny and Ausubel 1993) although is not described as such in the literature.
- SSR (microsatellite) genotyping relies on sequence information to design PCR primers flanking hyper-variable regions of non-coding repetitive DNA (Tautz 1989). SSR markers are co-dominant, and can have multiple alleles at one locus, meaning that a relatively small number of markers can provide high resolution genotyping, reducing the potential for inconsistencies between laboratories (Cooke and Lees 2004). Owing to the small size differences between alleles (often as little as 2 bp), fragment sizing is generally by capillary electrophoresis (Li *et al.* 2010). SSR markers for *P. infestans* have been developed following the increasing availability of *P. infestans* genome sequence data in the late 1990s and early 2000s (Cooke and Lees 2004; Knapova and Gisi 2002; Lees *et al.* 2006; Li *et al.* 2010). Owing to their ease of use, replicability, and good discriminating power, a set of 12 standard SSR markers is now the system favoured by Euroblight for internationally comparable characterisation of *P. infestans* populations (Li *et al.* 2013).

### **3.1.5 Choice of markers in the present study**

The decision to use SSR markers rather than AFLP, RFLP or Allozyme markers was straightforward, on account of ease of use and reliability of results. Mitochondrial haplotype analysis would have contributed additional information, but was deemed unnecessary given the resolution which can be obtained from SSR markers. Finally, a 12 SSR marker system has been



proposed by Li *et al.* (2013) as a standard system for *P. infestans* taxonomy. Thus, this system was adopted in the present study.

A conscious decision was made early on in the project not to investigate fungicide sensitivity, as it was not believed to be of great relevance to the primary aim of investigating host specialisation on tomato. In contrast, mating type and physiological race (in terms of aggressiveness on tomato and potato host leaflets) were clearly of relevance, and tests of these parameters were planned to take place in 2013. Unfortunately, an incubator malfunction meant that most of the isolates collected over the three years were killed, so phenotype tests requiring live cultures were not possible in most cases. In light of this fact, and the fact that optimising the SSR marker protocol took longer than expected, the decision was taken to focus solely on SSR genotype in this study.

### 3.1.6 Analysing marker data

#### 3.1.6.1 Software

*Phytophthora infestans* exhibits variable ploidy (in the present study, isolates with two, three, and four alleles at some loci were collected). This complicates data analysis, as most genetic analysis software is designed to handle data either from diploids, or from polyploid datasets where all individuals are of the same ploidy (Clarke 2011). The R packages *Polysat* (Clark and Jasieniuk 2011) and *Poppr* (Kamvar *et al.* 2014) were designed specifically for working with polyploid data, and contain implementations of the “Bruvo” distance measure, an algorithm for calculating genetic distance between polyploid SSR genotypes with allele copy-number ambiguity (Bruvo *et al.* 2004).

#### 3.1.6.2 Genetic distance calculation

Bruvo-distances are a microsatellite based measure of genetic distance between individuals, which may be of different ploidy. Differences in microsatellite repeat number are assumed to be due to slipped-strand mispairing during DNA replication (Bruvo *et al.* 2004). The likelihood of a given mutation is assumed to be inversely proportional to the number of repeat-unit differences, according to the formula:

$$m_x = 2^{-|x|}$$

Where  $x$  is the number of repeat-units by which the two alleles differ, and  $m_x$  is the probability of an  $x$  unit mispairing. The value of  $m_x$  will always lie between 0 and 1. The difference,  $d_a$ , between two alleles, is calculated as:

$$d_a = 1 - m_x$$

The genetic difference between two individuals at a given locus ( $d_l$ ) is defined as the minimum sum of all inter-allele differences divided by the ploidy level.

Where the ploidy levels of the two individuals are different, “virtual alleles” are added to the individual with lower ploidy to equalise the ploidies. Several options exist for assigning values to these virtual alleles (Bruvo *et al.* 2004), but *Polysat* uses the simplest of these, i.e. assigning a value of  $\infty$  to the virtual allele (Clarke 2013). This means that the value of  $x$  for any virtual allele - real allele pairing is equal to zero, and therefore the respective difference is equal to one:

$$\begin{aligned} d_a &= 1 - m_x = 1 - 2^{-|x|} \\ &= 1 - 2^{-\infty} = 1 - 0 = 1 \end{aligned}$$

The overall genetic distance between two individuals is the mean of the distances between the individuals at all loci.

### 3.1.6.3 Correlation of geographical and genetic distance

The Mantel test measures the strength of correlation (the Mantel Statistic,  $r$ ) between two distance matrices by performing a test for correlation between the values at equivalent positions in both matrices. The significance of this correlation is subsequently estimated by calculating multiple additional values of  $r$  with the entries in one of the matrices randomly rearranged, and comparing the “real” value of  $r$  to the values obtained by permuting the entries in the matrix. If a significant correlation exists between the values in the two matrices, the “real” value of  $|r|$  should be significantly greater than the values calculated from the permuted matrices (Manly 2004).

### 3.1.7 Aims of study

It was hypothesised that there might be distinct tomato and potato-specialised populations in line with the situation in other regions (discussed in **Chapter 1**). Since it has been suggested that gardens could be an important source of inoculum for commercial potato crops (Ball and Stevenson 2012), understanding more about the structure of *P. infestans* populations in these settings is vital.

This study was conducted to compare populations of *P. infestans* from tomato and potato for genetic variation at SSR (microsatellite) loci and to examine the diversity of *P. infestans* genotypes present in gardens, as distinct from commercial potato crops, in Britain between 2011 and 2013.

## 3.2 Materials and Methods

### 3.2.1 Isolation of *P. infestans* from Gardens

*P. infestans* samples were obtained from private gardens and allotment sites throughout Britain by appealing through several gardening websites and magazines and by directly contacting allotment organisations or public administrators in 300 major British cities and regions. In 2011 and 2012, *P. infestans* samples were sought from tomato only. In 2013, samples were sought from both tomato and potato crops. Responding groups and individuals were asked to mail fresh, otherwise healthy leaflets bearing small lesions to the authors.

Upon receipt, samples were placed in a 9 cm diameter Petri dish lined with damp paper and incubated at cool room temperature (15-20 °C) in diffuse natural light for approximately 24 hours. Once sporulation was observed, an agar wedge was used to transfer sporangia to a plate containing Rye A medium (Caten and Jinks 1968) amended with 25 mg L<sup>-1</sup> of rifampicin and ampicillin (both Bio Basic Canada Inc.) and 50 mg L<sup>-1</sup> of nystatin (Sigma Aldrich). The cultures were incubated in darkness at 18 °C in order to grow mycelium from which DNA was extracted. For some isolates, sporangia would not germinate on agar, and in this case DNA was extracted directly from infected plant material.

In 2011 and 2012 respectively, 15 and 36 *P. infestans* cultures were isolated from the samples received. In 2013, 25 cultures were isolated from potato, and 43 from tomato. In all years, most isolates came from England and Wales with very few from Scotland. Otherwise, the geographical distribution of isolate origins was reasonably even. The geographical distribution of isolate origins in the 2013 tomato- and potato-hosted samples was similar (**Figure 3.1**). 10 isolates were sent to the authors in response to the sample appeal without a note of their geographical origin or a means of contacting the sender. These 10 isolates were included in all analyses except the Mantel test for correlation between geographical and genetic distance.

In addition to the samples collected as part of this investigation, the following reference isolates of known clonal lineage were included: 2010\_8106A (23\_A1), 2006\_3928A (13\_A2), 2006\_3984C (1\_A1), 2006\_3888A (2\_A1), and 2006\_4232E (8\_A1). Reference isolates were supplied by Dr David Cooke from the collection held at the James Hutton Institute, Invergowrie, Dundee, UK. Reference isolate genotypes as determined in the present study were compared with published genotypes in order to standardise allele calling. For comparative purposes survey data from commercial potato crops for the same period, collected as part of national *P. infestans* surveys sponsored by the Potato Council Ltd, were used in the analyses (D.E.L. Cooke, James Hutton Institute, unpublished data).

### 3.2.2 DNA Extraction

Approximately 100 mg of mycelium scraped from the surface of an agar plate (or alternatively 100 mg of infected plant material) was placed in a sterile collection tube and freeze-dried. DNA extraction was carried out using DNEasy Plant Mini Kits (Qiagen) according to the manufacturer's instructions.

### 3.2.3 PCR Amplification

PCR was carried out using Qiagen Multiplex PCR kits (Qiagen) with primer pairs for 12 SSR loci described by Li *et al.* (2013). Their protocol was modified to use WellRED dyes (Beckman Coulter) in two six-plex panels rather than one twelve-plex panel (**Table 3.1**). The final reaction concentration of primer pair SSR4 was increased from 0.05  $\mu\text{M}$  to 0.1  $\mu\text{M}$  (each primer). PCR was carried out in a total volume of 12.5  $\mu\text{L}$  using a PTC-100 Thermocycler (MJ Research). The reaction mixture consisted of: 6.25  $\mu\text{L}$  Qiagen Multiplex PCR Master Mix (Qiagen), 0.3125  $\mu\text{L}$  each primer (**Table 3.1**), 1.5  $\mu\text{L}$  template DNA (6 ng  $\mu\text{L}^{-1}$ ) and 1  $\mu\text{L}$  water. PCR conditions were as follows: 95 °C for 15 minutes, followed by 30 cycles of 95 °C for 20 s, 58 °C for 90s, and 72 °C for 60 s, and a final extension at 72 °C for 20 minutes. PCR amplification and fragment sizing were carried out at least twice for all samples as a check against failed amplification or fragment sizing errors.

### 3.2.4 Fragment Sizing

Fragment sizing was carried out using a CEQ 8000 genetic analysis platform (Beckman Coulter), according to the manufacturer's instructions. The samples were run using the CEQ 8000 *Frag-3* programme, which consisted of a 30 second injection at 2 kV and a 35 minute capillary run at 6 kV, 50 °C. Alleles were called manually using the nomenclature described by Li *et al.* (2013). The fragments produced by some primer pairs were consistently larger with the present protocol than sizes published by Li *et al.* (2013). The size of any deviation (0 - 24 bp according to locus) was established by comparing the fragment sizes obtained using the reference samples with published fingerprints and the appropriate correction made when recording sample fragment sizes (**Table 3.1**).

**Table 3.1: Details of SSR marker primers used**

The final concentrations, WellRED dye labels, and panel groupings of the 12 microsatellite primers used in this study (after Li *et al.* (2013)). The concentration of primer SSR4-F was increased from 0.05 to 0.1  $\mu\text{M}$ . Fragments detected in this study were generally larger than those published by Li *et al.* (2013), and the deviation from the original size is indicated. The fragment sizes remained consistent over multiple PCR and fragment sizing runs. Primer stocks were prepared at the concentrations indicated so that the same volume of each could be added to the mastermix whilst retaining the desired primer ratio.

Panel 1					Panel 2				
Primer Name	WellRED Label	Product Size Deviation (bp)	Final Conc.	Primer Stock Conc. ( $\mu\text{M}$ )	Primer Name	WellRED Label	Product Size Deviation (bp)	Final Conc.	Primer Stock Conc. ( $\mu\text{M}$ )
PiG11	D3	0	(F) 0.05	2	D13	D4	0	(F) 0.16	6.4
			(R) 0.05	2				(R) 0.05	2
Pi04	D2	24	(F) 0.3	12	SSR2	D2	17	(F) 0.05	2
			(R) 0.3	12				(R) 0.05	2
Pi4B	D3	1	(F) 0.05	2	Pi70	D3	17	(F) 0.05	2
			(R) 0.05	2				(R) 0.05	2
SSR3	D2	0	(F) 0.1	4	SSR6	D3	19	(F) 0.05	2
			(R) 0.1	4				(R) 0.05	2
SSR8	D4	21	(F) 0.3	12	Pi63	D3	1	(F) 0.05	2
			(R) 0.3	12				(R) 0.05	2
SSR4	D4	20	(F) 0.1	4	SSR11	D2	0	(F) 0.05	2
			(R) 0.1	4				(R) 0.05	2

### 3.2.5 Datasets

Four datasets were used for different parts of the analysis:

The *Full Dataset* was a mixed-ploidy dataset composed of all samples collected in this study.

The *Full Diploid Dataset* was derived from the *Full Dataset*. Where more than two alleles were present at a locus (trisomic, triploid or tetrasomic or tetraploid isolates), the mid-sized allele(s) were removed, producing an artificially diploid dataset (as described by Li *et al.* (2012) and Lo *et al.* (2009))

The *Clone-Corrected Dataset* was derived from the *Full Dataset*. Where multiple isolates had identical SSR genotypes, only one was retained (generally, the first isolate representative of a genotype to be received was used). However, in 2013, in instances where a SSR genotype was

present in both the potato- and tomato-hosted sample, an isolate from each host population was retained.

The *Clone-Corrected Diploid Dataset* was derived from the *Full Diploid Dataset* by removing duplicate isolates as for the *Clone-Corrected Dataset*.

*Reference* genotypes of published named clonal lineages (Li *et al.* 2012) were included for comparison in some analyses.

### **3.2.6 Assignment of Clonal Lineages**

#### *3.2.6.1 Calculation of genetic distances*

The *Full Dataset* plus 96 *Reference* genotypes were used to establish whether or not samples collected as part of the present study belonged to known clonal lineages. Inter-individual “Bruvo” distances (Bruvo *et al.* 2004) between all genotypes were calculated using the *meandistance.matrix* function of the package *Polysat* (Clark and Jasieniuk 2011) on the R statistics platform (R Core Team 2014).

The resulting distance-matrix served as the input to the *assignClones* function in *Polysat*, in order to group the study samples with the 96 *Reference* genotypes. The grouping threshold was set at 0.15 because this was found to be the level at which the 96 *Reference* genotypes would group together within their designated clonal lineages without grouping with *Reference* isolates from other clonal lineages. Additionally, the *Phytophthora-ID 2.0* website (Grünwald *et al.* 2014) was used to identify clonal lineages.

### **3.2.7 Detection of Underlying Genetic Structure**

#### *3.2.7.1 Population diversity*

The Shannon-Wiener diversity was calculated for each sample population. The unique isolates were treated as a single, homogeneous category and similarly, the rarest unidentified clonal lineages (Unknown-2, Unknown-5 and Unknown-6) were combined into a single category. Fisher’s exact test was used to test for significant differences between populations.

#### *3.2.7.2 Population differentiation*

Principal Coordinates Analysis was carried out using the *cmdscale* function of the *stats* package in R (R Core Team 2014) to generate two principal components from the Bruvo distance table produced from the *Full Dataset*.

The *Clone Corrected Dataset* served as the input for an Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) using the R package *Poppr* (Kamvar *et al.* 2014) in order to determine the proportion of genetic variability between and within populations.

### 3.2.7.3 Population structure

The *Clone-Corrected Diploid Dataset* served as the input for *STRUCTURE 2.3.4* (Pritchard *et al.* 2000), which used a Markov Chain Monte Carlo clustering algorithm to infer the number of reproductively-isolated sub-populations (K) and to probabilistically assign each isolate to a sub-population. A 100,000 iteration burn-in period was followed by 1,000,000 iterations. K was allowed to vary from 1 to 10. The allele-frequency parameter  $\lambda$  was set to 1, and the *admixture model* was assumed. This simulation was replicated 20 times. The output from these replicate runs served as the input for *STRUCTURE Harvester* (Earl and vonHoldt 2012), which was used to ascertain the mean value of K across the 20 simulation runs using the method of (Evanno *et al.* 2005). Subsequently, *CLUMPP* (Jakobsson and Rosenberg 2007) was used to find optimum clustering of individual isolates into the K clusters across the 20 replicate *STRUCTURE* outputs, using the *Greedy option 2* algorithm and 200,000 repeats. *Microsoft Excel* was used to display the results graphically.

### 3.2.7.4 Geographical structuring

A Mantel Test was conducted to test whether the genotype of samples was correlated with the location from which they were collected. A Euclidian geographical distance matrix was calculated for all 67 isolates collected in 2013 for which the collection location was known. The distance matrix was calculated using the *dist* function of the *stats* package in R, having converted the GB postcodes supplied with the isolates to X-Y coordinates (UK Eastings and Northings) using the “Doogal” online geocoding tool (Bell 2015). The (Bruvo) genetic distance matrix generated using *Polysat* was edited to include only the 67 isolates for which location data was available. The *mantel* function of the *vegan* package (Oksanen *et al.* 2015) in R (R Core Team 2014) was used to test for significant Pearson Correlation between the two matrices, using 100,000 permutations.

## 3.3 Results

### 3.3.1 Receipts from Surveys

The survey strategy adopted in 2011 and 2012 (depending largely on magazine adverts) proved to be only moderately effective, resulting in the receipt and successful isolation of 15 and 36 samples respectively. The more pro-active sampling strategy adopted in 2013 generated more samples (**Table 3.2**). In all years, samples were mostly from England and Wales, with few coming from Scotland (**Figure 3.1**). In 2013, the geographical distribution of samples from tomato and potato was similar (**Figure 3.1c-d**). In addition to the isolates plotted on the map, a single isolate sent from Jersey and 10 isolates sent without details of their collection location were received.

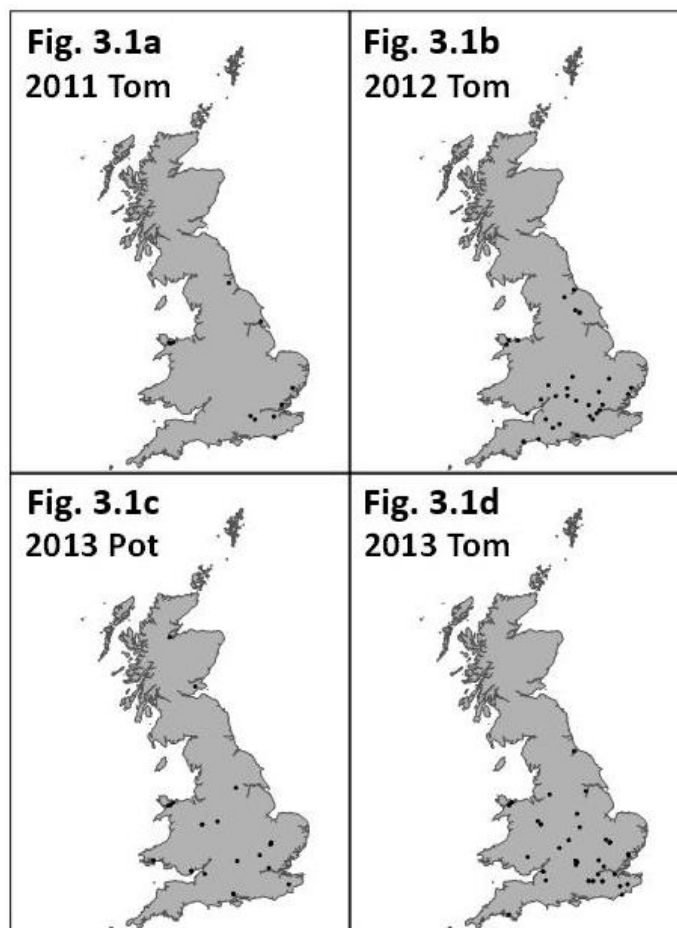
### 3.3.2 Population Composition

Genotypes were obtained from 119 isolates (Supplementary Data 2). Four known clonal lineages (13\_A2, 6\_A1, 8\_A1, 23\_A1 and 1\_A1) were identified amongst the *P. infestans* isolates collected from gardens. A high proportion (36%) of the garden-derived isolates were unique genotypes that did not group with any other isolates and are identified here as “Unique” (**Table 3.3**). In total, 106 distinct genotypes were present amongst the 119 isolates collected.

Seven groups of isolates were identified as clonal lineages (i.e., the Bruvo distance between the isolates was <0.15) that were distinct from all named clonal lineages in the available databases (D.E.L. Cooke, James Hutton Institute, unpublished data; Grünwald *et al.* 2014; Li *et al.* 2013). Furthermore, the isolates making up these groups were collected from distinct outbreaks in well separated geographical locations. Accordingly, these groups were tentatively identified as newly discovered clonal lineages and named “Unknown\_1” through to “Unknown\_7”.

The *P. infestans* population on commercial potato crops in Britain was dominated by the 13\_A2 and 6\_A1 clonal lineages during the period of this study, with these clonal lineages together making up 88%, 86% and 87% of the sample in 2011, 2012 and 2013 respectively (**Table 3.3**) (D.E.L. Cooke, James Hutton Institute, unpublished data). In the tomato-hosted samples from gardens for 2011, 2012 and 2013, the percentages of 13\_A2 and 6\_A1 together were 33%, 11% and 25%, respectively (**Figure 3.2a-c**). The 2013 potato sample from gardens contained 48% 13\_A2 and 6\_A1 isolates (**Figure 3.2d**). Isolate 23\_A1 made up 20% and 14% of the samples collected from tomato in 2011 and 2012 respectively (**Figure 3.2a-b**), compared to a single isolate (0.23 %) in 2011 and four isolates (0.56 %) in 2012, in the sample from commercial potato crops (D.E.L. Cooke, James Hutton Institute, unpublished data). In 2013, 23\_A1 was absent from both garden samples (**Figure 3.2c-d**) and from the commercial potato sample (D.E.L. Cooke, James Hutton Institute, unpublished data).





**Figure 3.1: Origins of *P. infestans* samples**

**(a)** 2012 tomato sample; **(b)** 2013 potato sample; **(c)** and 2013 tomato sample; **(d)**. Not shown on maps are: four additional isolates in 2011, four in 2012, one in 2013 (potato), and one in 2013 (tomato) sent to the authors from unrecorded locations in Britain; also one isolate sent from Jersey in 2012.

**Table 3.2: Samples received during the course of the study**

The number of usable samples is the number of distinct samples which were confirmed to be *P. infestans* and from which DNA was extracted and a genotype obtained. The total number received is the total number of samples, including samples on which *P. infestans* could not be detected, and multiple samples collected from the same host, at the same location, at the same time. The number of samples received was not recorded in 2011 or 2012.

Year	Host	No. Received	No. Usable
2011	Tomato	-	15
2012	Tomato	-	36
2013	Tomato	93	43
	Potato	57	25
Total		-	119

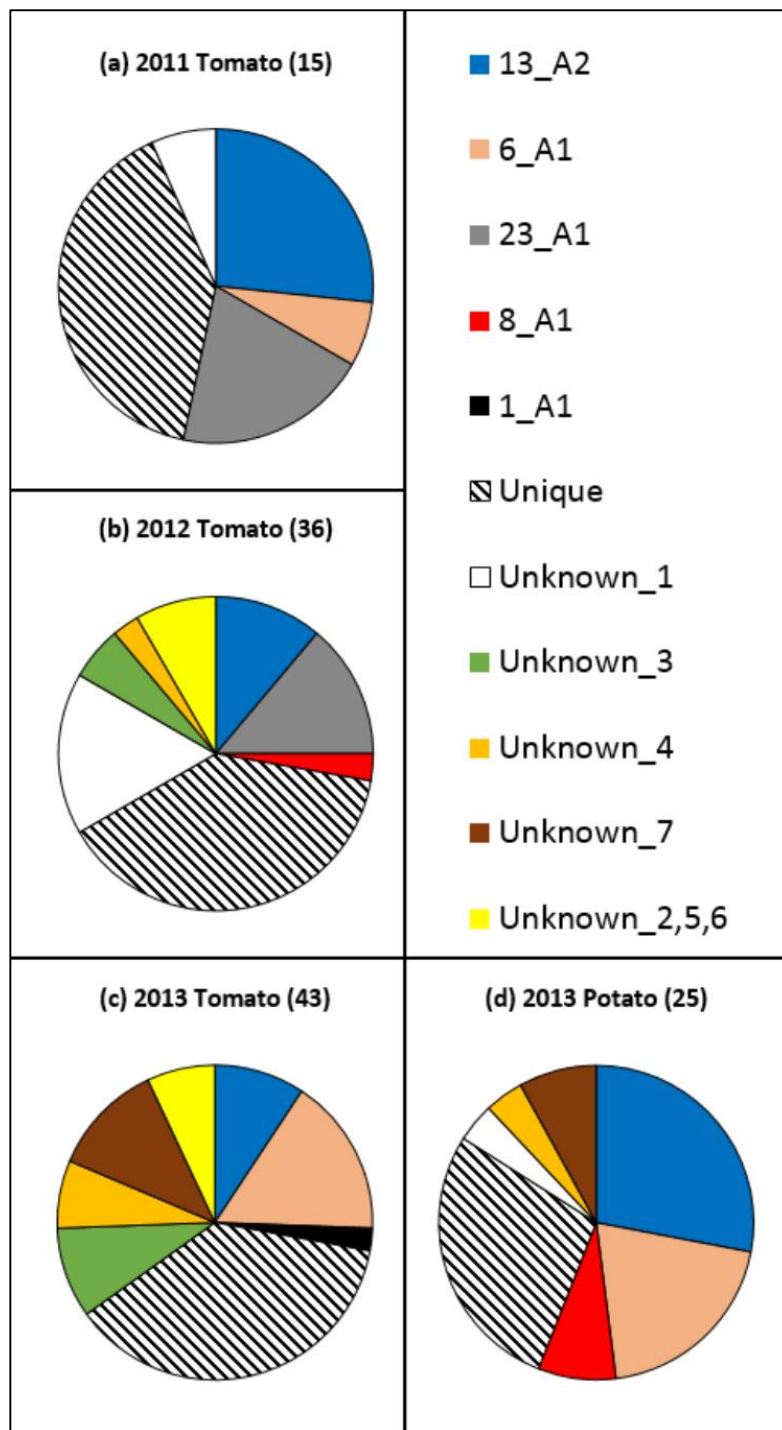
In all years, garden samples contained a higher proportion of “Unique” isolates compared to the corresponding sample from commercial potato crops (**Table 3.3**), with such isolates making up 36% of the garden sample (mean of all years, both hosts), compared to 7% of the commercial potato sample (mean of all years).

Highly significant ( $p < 0.001$ ) differences were found between the frequencies of clonal lineages from commercial crop populations (D.E.L. Cooke, James Hutton Institute, unpublished data) and garden populations in all years (**Table 3.3**). Despite the presence of different clonal lineages in the potato- and tomato-hosted populations collected from gardens in 2013 (**Figure 3.2c-d**), a Chi-squared test for difference in lineage frequency between the two populations did not indicate a significant difference ( $p = 0.107$ ).

**Table 3.3: Lineage diversity of sample populations**

The Shannon-Wiener diversity index of the garden-derived samples collected for this study and samples mostly taken from commercial crops characterised at the James Hutton Institute (D.E.L. Cooke, James Hutton Institute, unpublished data). N indicates the number of isolates in the sample. The percentages in each population are given for the two most common clonal lineages (13\_A2 and 6\_A1) and “Unique” isolates. Within each year, samples with different letters in the Group column were significantly different ( $p < 0.001$ ).

Setting	Year	Population Host	N	Shannon-Wiener	% 13_A2 & 6_A1	% Unique	Group
Garden	2011	Tomato	15	1.40	33	40	a
Commercial	2011	Mainly Potato	436	0.74	88	7	b
Garden	2012	Tomato	36	1.75	11	39	a
Commercial	2012	Mainly Potato	716	1.08	86	7	b
Garden	2013	Tomato	43	1.81	25	36	a
Garden	2013	Potato	25	1.70	48	28	a
Commercial	2013	Mainly Potato	219	1.05	87	8	b
Mean Garden	All	Both hosts	119	2.00	27	36	a
Mean Commercial	All	Mainly Potato	1371	1.04	87	7	b



**Figure 3.2: Clonal lineage composition of all *P. infestans* sample populations**

The distribution of clonal lineages within *P. infestans* samples collected from British gardens and allotments from tomato hosts (**a-c**) and potatoes (**d**) collected for this study. Sample size is indicated in parentheses in the title of each graph. Segments are shaded by *P. infestans* clonal lineage. Fisher's exact test indicates significantly ( $p < 0.001$ ) different distributions for all comparisons between study samples (**a-d**) and the corresponding sample collected from commercial potato crops in each year (D.E.L. Cooke, James Hutton Institute, unpublished data, not shown) but not between the 2013 tomato and potato samples from gardens (**c-d**) where  $p = 0.107$ .

### 3.3.3 Population Structure

#### 3.3.3.1 Correlation between geographical and genetic distances

A Mantel test performed on 67 potato and tomato isolates from the 2013 sample did not detect any significant correlation between geographical and genetic distance between isolates ( $r = 0.057$ ,  $P = 0.165$ ).

#### 3.3.3.2 Analysis of Molecular Variance

AMOVA (**Table 3.4**) showed that 98% of the genetic variance in the clone-corrected 2013 sample existed within populations, and host population accounted for only 2% of genetic variation. The association between host and genotype was not significant ( $P = 0.071$ )

#### **Table 3.4: AMOVA Results**

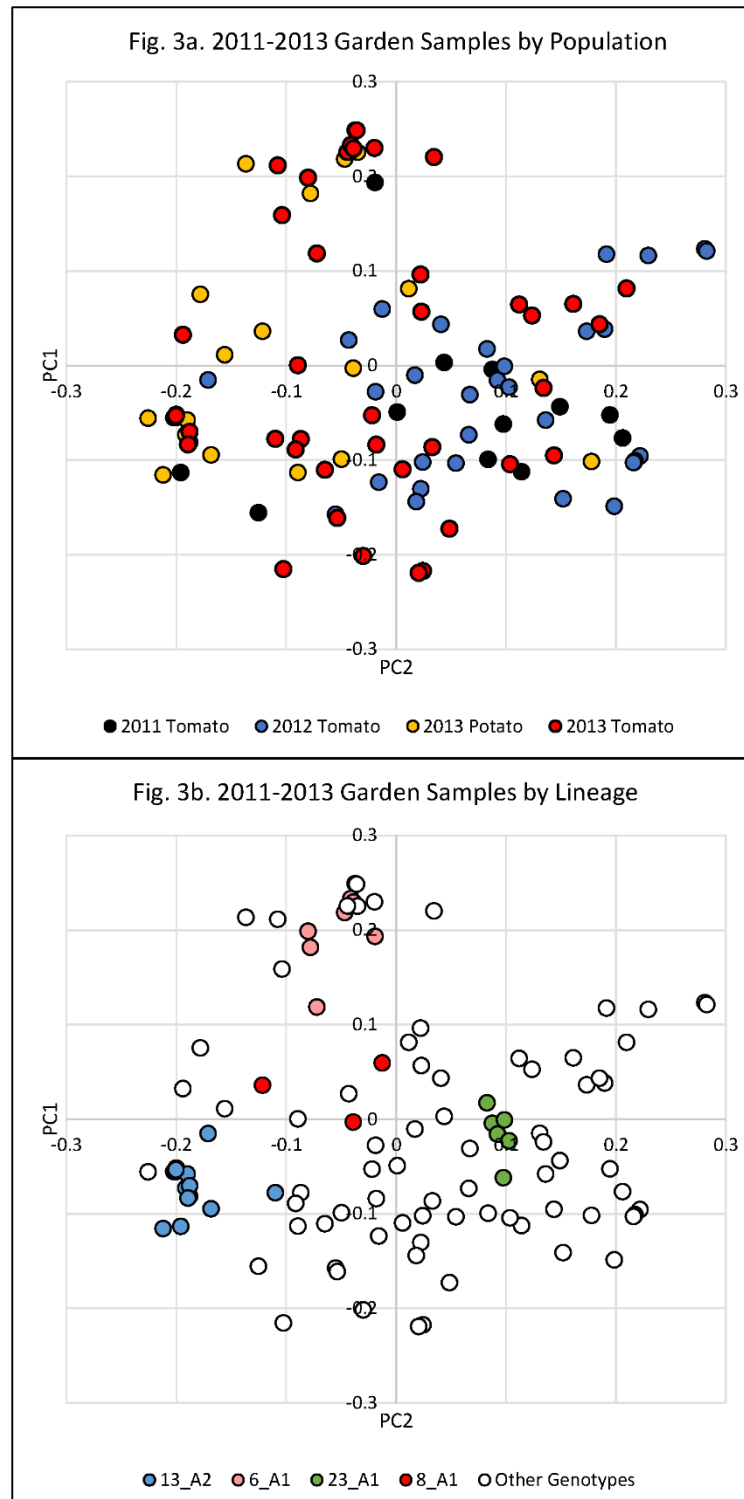
Summary of the results of Analysis of Molecular Variance (AMOVA) conducted on the 2013 isolates from the *clone-corrected dataset*, indicating the percentage of genetic variation attributable to inter-population differences between potato- and tomato-hosted isolates and between individual isolates within populations.

Source of Variation	% of Total	P
Between Populations	2.0	0.071
Within Populations	98.0	
Total	100	

Principal Coordinates Analysis did not reveal clustering of isolates by host in 2013, although some degree of separation of isolates by year was apparent (**Figure 3.3a**), and clustering by clonal lineage was clear (**Figure 3.3b**).

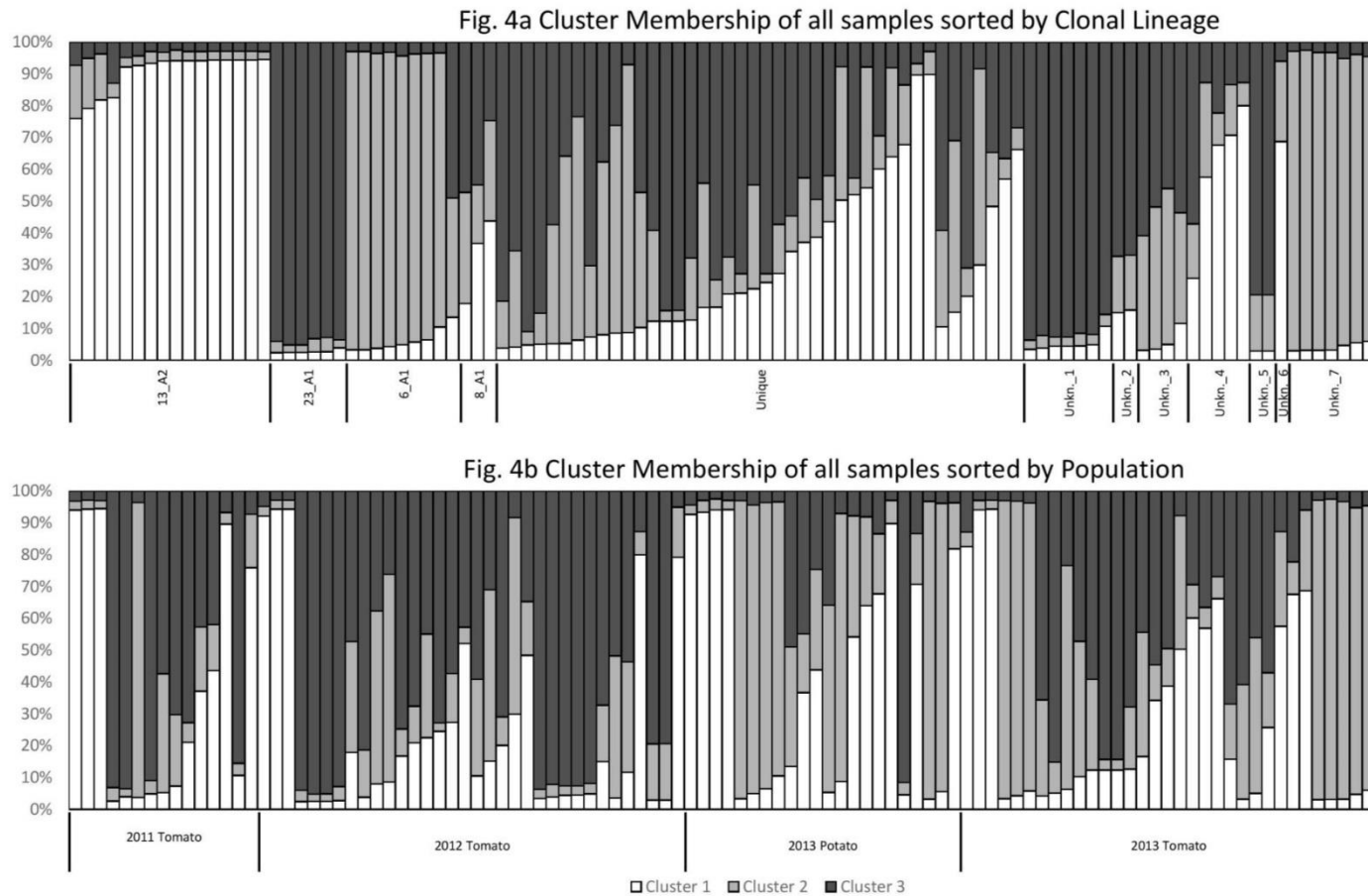
#### 3.3.3.3 STRUCTURE analysis

Clustering using STRUCTURE identified  $K=3$  clusters. These clusters were not clearly associated with sample populations (**Figure 3.4**) and isolates belonging to each of the three clusters were distributed throughout the sample populations, although there may be a slight tendency for potato-derived isolates to belong to Cluster 3 (**Figure 3.4b**). It was clear that isolates from the same clonal lineages shared similar patterns of cluster membership (**Figure 3.4a**), and that the tomato-associated lineage 23\_A1 was part of Cluster 3.



**Figure 3.3: Principal co-ordinate plots showing relationship between SSR genotypes**

Plots of the first two principal coordinates resulting from classical multidimensional scaling of inter-isolate genetic distances (Bruvo) calculated from combined data of 12 SSR markers on *P. infestans* isolates from gardens and allotments with symbols showing each population sampled (a) and clonal lineage identified (b). “Other Genotypes” includes all *Unique* isolates and less common named clonal lineages.



**Figure 3.4: STRUCTURE analysis output**

Markov Chain Monte Carlo clustering for  $K=3$  clusters of blight isolates. Each isolate is represented by a vertical bar and the proportion (%) of identity to each cluster is represented by dark grey, light grey and white bars. Optimal clustering of individuals was obtained using CLUMPP (Jakobsson and Rosenberg 2007) from 20 STRUCTURE runs of 1,000,000 iterations following a 100,000 iteration burn-in period. **(a)** shows genotypes grouped by sample population, showing possible slight association between *P. infestans* genotypes belonging to Cluster 3 and tomato host. **(b)** shows genotypes grouped by clonal lineage, showing association with cluster membership. At the time that this analysis was conducted, only one of two isolates of lineage Unknown-6 had been received, and Unknown-6 had not been identified as a recurrent lineage. The first isolate to have been received is indicated, however.

## 3.4 Discussion

### 3.4.1 Isolates identified

119 usable samples were received in all (Table 3.2). The number of samples received may have been reduced by a cold and dry early season in 2011 (in the Midlands and parts of Wales), which was not conducive to late blight outbreaks, although the exceptionally wet summer of 2012 almost certainly increased the success of the *P. infestans* survey (Met Office 2015). The higher proportion “Unique” isolates and lower proportion of 13\_A2 and 6\_A1 isolates recovered in the present study in comparison with national surveys conducted by the James Hutton Institute is discussed below, but the greater proportion of isolate 23\_A1 and presence of seven previously unidentified lineages was also noteworthy.

#### 3.4.1.1 23\_A1

Isolate 23\_A1 was first recorded by researchers at the James Hutton Institute in 2007 (three isolates from tomato in Southern England) and then next detected in 2009 (eight isolates from commercial potato crops throughout Britain) and was scattered throughout Britain in 2010 (Cooke *et al.* 2014). This isolate was informally reported by Cooke *et al.* (2009) to be associated with tomato in Italy, and had been recovered from tomato, petunia and potato in Britain. However, by 2011 it had become rare in Britain again, with one isolate (0.23%) in the commercial sample in 2011, four isolates (0.56%) in 2012. In the present study, 23\_A1 made up a substantial proportion of the isolates collected (from tomato) in 2011 and 2012, with three isolates (20%) in 2011 and five isolates (14%) in 2012. Taken together, these facts do point towards an association between the 23\_A1 lineage and tomato host. The complete absence of 23\_A1 from both the potato and tomato sample collected in 2013 as part of the present study, and the 2013 sample of commercial crops by the James Hutton Institute (D.E.L. Cooke, James Hutton Institute, unpublished data) is puzzling, but does not necessarily corroborate or contradict the suggestion that 23\_A1 is tomato-associated.

#### 3.4.1.2 Novel clonal lineages

Seven clonal lineages (“Unknown-1” to “Unknown-7”) were identified as being at least as dissimilar to any of the reference genotypes as the different reference lineages were to each other. It is surprising that recurrent clonal lineages were found at locations throughout Britain without having been reported previously. However, the list of reference genotypes was not exhaustive. Furthermore, these seven lineages may be unable to infect potato cultivars with genetic resistance, or to tolerate fungicide sprays, and may therefore be uncommon or absent from

commercial plantings. Since the majority of *P. infestans* sampling in Britain to date has been in commercial fields (Cooke *et al.* 2014) it is plausible that these lineages have evaded detection.

### 3.4.2 Comparison of diversity of garden and commercial crop populations

Amongst the 119 samples collected, diversity was high, with 106 distinct genotypes identified. The Shannon-Wiener diversity index of 2.0 was almost double that (1.04) of the much larger sample collected from commercial fields during the same period. The true diversity of the garden-derived samples may have been higher still, because dissimilar genotypes were combined in the “Unique” category. Also, the small sample size taken in this study limits detection of rarer types. The greater diversity found in gardens in Britain is in agreement with findings of Drenth *et al.* (1993b), who surveyed Dutch *P. infestans* populations and found that the A2 mating type was more common in allotment-derived samples (53%) than in those from commercial fields (12%) over the same period. Drenth *et al.* (1993a) also found greater RFLP genotype diversity in samples collected from allotments and community gardens than commercial potato fields in the Netherlands.

#### 3.4.2.1 Role of sexual recombination in gardens

The higher diversity index, presence of seven apparently novel clonal lineages, and high proportion (36%) of “Unique” isolates, indicates that the structure and dynamics of *P. infestans* populations in gardens and allotments may be rather different to the situation on commercial potato crops. Cooke *et al.* (2014) suggest that “Unique” isolates may be the recombinant progeny of matings between A1 and A2 genotypes, indicating that matings and genetic recombination may be more common in gardens than in commercial crops.

#### 3.4.2.2 Survival of less virulent and fungicide-sensitive isolates

Aside from the possibility that diversity arises from matings, possible explanations for higher diversity of *P. infestans* genotypes in gardens and allotments may include the fact that fungicides are less often used by gardeners than by commercial farmers, and this may allow fungicide-susceptible *P. infestans* genotypes to flourish in these settings. Furthermore, a wider range of varieties are grown in gardens than in commercial systems, including many “heritage” varieties developed before blight-resistance was a common breeding objective. Gardeners may therefore grow crops of susceptible potato varieties unprotected by fungicide sprays, again providing an environment in which less virulent *P. infestans* genotypes can flourish in addition to those which infect commercial crops. Infected potato seed tubers from a larger number of different sources may come together in allotment sites, facilitating the recombination of *P. infestans* genotypes through matings. This could explain the high proportion of unique isolates encountered in these



settings. Finally, biosecurity measures such as removal of all “volunteer” tubers and proper disposal of outgrades are likely to be less consistent in garden and allotment settings, reducing the rate of stochastic extinction of genotypes from one season to the next.

### 3.4.3 Lack of geographical population structuring

The majority of isolates collected for this study in 2013 came from England and Wales, with few from Scotland (**Figure 3.1**). In recent years, England and Wales have had *P. infestans* populations more similar to each other than to the population in Scotland (Cooke *et al.* 2013; Cooke *et al.* 2015), so the present survey may have overlooked the larger-scale geographical variation in the British *P. infestans* population. Still, the lack of any significant detectable correlation between isolate origin and genotype is surprising. This situation may arise from the (postulated) more sexual, less clonal, population in gardens. Assuming that asexually propagated clonal lineages play a less important role in garden populations, less dominance of certain lineages (i.e., certain allele combinations) in large areas would be expected, with “Unique” isolates (having genotypes composed of randomly assorted alleles) dominating instead. This appears to be the case in the samples collected in the present study.

### 3.4.4 Failure to detect host specialisation with SSRs

This study using SSR markers found little evidence of host-specialisation, despite revealing a broad palette of genetic variation in *P. infestans* populations from British gardens. The possible clustering of tomato and potato isolates identified by STRUCTURE analysis over the three years suggested that there is a small effect of host species on intra-lineage *P. infestans* variability in Britain, although no such effect was detected statistically by AMOVA in the 2013 samples. Comparison of lineage frequencies in tomato- and potato-hosted samples from gardens in 2013 did not identify any significant difference between the two hosts.

Where previous studies (**Table 1.1**) have examined clonal lineage using molecular marker systems (SSR or RFLP) they have generally found a clear distinction between the lineage frequencies in tomato- and potato-hosted populations (Brommonschenkel 1988; Garry *et al.* 2005; Knapova and Gisi 2002; Lebreton and Andrivon 1998; Oyarzun *et al.* 1998; Peters *et al.* 2014; Reis *et al.* 2003; Wangsomboondee *et al.* 2002). Of the studies using molecular marker systems, three found higher diversity on tomato (Garry *et al.* 2005; Lebreton and Andrivon 1998; Wangsomboondee *et al.* 2002), three found higher diversity on potato (Danies *et al.* 2012; Reis *et al.* 2003; Statsyuk *et al.* 2014), and two found a similar diversity on both hosts (Oyarzun *et al.* 1998; Peters *et al.* 2014). In the case of the present SSR study, Shannon diversity of potato- and tomato-hosted populations of *P. infestans* from comparable (garden) settings was similar.

Chowdappa *et al.* (2013) found no difference in diversity between samples, but in contrast to the present study, there was low overall diversity as all isolates belonged to the 13\_A2 lineage. Knapova and Gisi (2002) found conflicting results between two different marker systems, with higher diversity among the potato isolates using AFLP markers, but higher diversity among tomato isolates when using SSR markers. However, the study used only two SSR markers, and other studies using larger numbers of SSR markers found that SSRs were consistent with other neutral markers (Chowdappa *et al.* 2013; Danies *et al.* 2012; Fry *et al.* 2013). It is noteworthy that many previous studies were conducted in tropical countries (**Table 1.1**) where a number of hosts may be present year-round (see below), and that tomato and potato samples often came from different geographical regions, weakening any conclusion as to the effect of host on sample composition (Garry *et al.* 2005; Peters *et al.* 2014; Wangsomboondee *et al.* 2002). In the present study, both potato and tomato samples were collected from throughout Britain, and no correlation between geographical origin and genotype was detected, which was unsurprising given the lack of population structure identified in other analyses.

#### **3.4.5 Phenotypic variation**

Differences in aggressiveness or virulence may exist within clonal lineages in Britain, as in other countries (Danies *et al.* 2012; Delgado *et al.* 2013; Fry *et al.* 2013; Garry *et al.* 2005; Knapova and Gisi 2002; Lebreton *et al.* 1999; Legard *et al.* 1995; Oyarzun *et al.* 1998; Vega-Sanchez *et al.* 2000). It is therefore unfortunate that accidental loss of most of the isolate collection meant that no phenotypic traits could be analysed in this study. The detached leaflet tests described in **Chapter 4** were carried out with some of the surviving *P. infestans* isolates collected during this survey, and included three isolates from tomato and one from potato. The potato-derived isolate infected tomato leaflets with the same or great frequency as the tomato derived isolates, although the rate of lesion expansion, and intensity of sporulation from lesions, was markedly lower. Clearly there is scope to examine the aggressiveness and virulence of tomato- and potato-hosted *P. infestans* isolates collected in Britain against susceptible tomato and potato differentials, and those carrying a variety of resistance genes.

There may be a biological reason for a lack of differentiation between tomato- and potato-hosted *P. infestans* populations in Britain. The British climate generally precludes *P. infestans* from overwintering on any host other than potato. In contrast to the situation in many other European countries, oospore-mediated infections in commercial plantings appear to be rare in Britain (Collins 2013; Cooke *et al.* 2014; Lees *et al.* 2012) indicating that overwinter survival by oospores in commercial crops is not likely. Therefore, in order to persist from year to year, *P. infestans* lineages must be capable of infecting potato, the only common overwintering host. A mirror situation was suggested by Le *et al.* (2008) as an explanation for the apparent absence of host

specialisation in Vietnam, where cropping cycles mean that tomato is the only available host for part of the year. However, it is notable that clear evidence of host specialisation exists in France and Switzerland (Knapova and Gisi 2002; Lebreton *et al.* 1999) and in Canada and the north of the USA (Danies *et al.* 2012; Fry *et al.* 2013; Peters *et al.* 2014) where a similar growing situation to Britain exists, so the lack of continuous presence of both hosts does not appear to preclude specialisation. Reis *et al.* (2003) suggested that apparent host-preference in Brazil could be the result of the fact that tomatoes are generally grown in warmer areas than potatoes. In the present study, however, the influence of geographical location (and by extension, climatic factors) on genotype was explicitly ruled out by the lack of any correlation between genetic and geographical distance.

### **3.5 Conclusions and recommendations**

No evidence of host specialisation or of geographical structuring in the *P. infestans* sample collected from British gardens was identified, beyond an apparent association between the lineage 23\_A1 with tomato. However, high diversity, including a high proportion of “Unique” isolates and novel clonal lineages, was identified. Phenotypic studies were not carried out, and would obviously be of value in determining whether host adapted genotypes do occur. This work highlights the need to continue efforts to educate and inform gardeners of the importance of late-blight prevention and control measures. It also emphasises the need to develop potato and tomato varieties with better late-blight resistance and promote them to gardeners.

## 4 Dissecting the *Phytophthora infestans* resistance of tomato cultivars with different *Ph*- gene combinations

### 4.1 Introduction

To date, three genes for *Phytophthora infestans* resistance, *Ph-1*, *Ph-2* and *Ph-3*, have been introgressed into commercially released tomato cultivars. *Ph-1* confers immunity to *P. infestans* race T<sub>0</sub> (Conover and Walter 1953). *Ph-2* confers rate-limiting partial resistance to a broad range of *P. infestans* isolates (Turkensteen 1973) and *Ph-3* confers strong resistance or immunity to many *P. infestans* isolates (Chunwongse *et al.* 2002). *Ph-1* was used in several cultivars including open-pollinated (OP) New Hampshire Surecrop, (Rich and Yeager 1957), Rockingham OP (Rich *et al.* 1962) and New Yorker OP (Robinson *et al.* 1967) and gave complete resistance to *P. infestans* race T<sub>0</sub>. However, within a few years of its introduction into horticultural varieties, *P. infestans* strains capable of overcoming the resistance conferred by *Ph-1* became widespread (Conover and Walter 1953). Accordingly, *Ph-1* is now widely considered to be a “defeated” resistance gene and is no longer used in tomato breeding programmes (Foolad *et al.* 2008; Nowicki *et al.* 2013). *Ph-2* and *Ph-3* offer useful levels of resistance to isolates of *P. infestans* common in most world regions (including Europe and the USA) and so are the main source of resistance in most blight-resistant cultivars currently grown. F<sub>1</sub> hybrid cultivars released recently in the USA that use these genes include Mountain Magic (Gardner 2008), Mountain Merit (Panthee and Gardner 2010), and Defiant (McGrath *et al.* 2013), all of which are heterozygous carriers of both *Ph-2* and *Ph-3*. Cultivar Iron Lady carries both genes in the homozygous state (McGrath *et al.* 2013), as does breeding line NC2-CELBR (Gardner and Panthee 2008), the resistant parent of Mountain Magic and Mountain Merit. Plum Regal carries heterozygous *Ph-3* (Randy Gardner, *pers. comm.*), and a number of older cultivars including Ferline F<sub>1</sub> and Fantasio F<sub>1</sub> (*unpublished data*, see **Chapter 2**), Legend OP (Savonen *et al.* 2008) and West Virginia '63 OP (Gallegly 1964) carry *Ph-2* in either the homozygous or heterozygous state.

More recent field trials (Brusca 2003; Hansen *et al.* 2014; Johnson *et al.* 2014; McGrath *et al.* 2012; McGrath *et al.* 2013; McGrath and LaMarsh 2014) as well as those described in **Chapter 2**, and anecdotal evidence from growers, points to far stronger resistance with a combination of both *Ph-2* and *Ph-3* genes than from either gene alone. Brusca (2003) additionally noted that only lines with *Ph-2* and *Ph-3* combined produced usable fruit in heavily infected field trials.

*P. infestans* is capable of overcoming isolate-specific resistance genes rapidly (see **Chapter 1**). Potato cultivars carrying single *R* genes were often overcome by new virulent *P. infestans* races

within a few years of release (Fry 2008b), as were tomato cultivars relying on *Ph-1* (Conover and Walter 1953). Accordingly, efforts are now underway to “stack” or “pyramid” multiple *P. infestans* resistance genes within potato cultivars, with the aim of providing more durable resistance. In tomato, there is currently little scope for stacking of major resistance genes, as only two (*Ph-2* and *Ph-3*) offer significant resistance against *P. infestans*. However, there is a recognition of the urgent need to identify additional resistance genes and QTLs (see **Chapter 5**) in order for breeders to incorporate more durable, multi-gene resistance into new cultivars (Foolad *et al.* 2008; Nowicki *et al.* 2013; St Clair 2010).

### *Hypotheses*

- 1) The defeated *Ph-1* gene may have some residual effect on one or more resistance components.
- 2) A combination of *Ph-2* and *Ph-3* will give stronger resistance (on at least one resistance component) than either gene alone.
- 3) Gene copy number affects strength of resistance, with homozygous resistance genes resulting in stronger resistance (for at least one resistance component).

The detached-leaflet experiments described in this chapter were designed in order to test the above three hypotheses using available cultivars or breeding lines known to carry the genes and *P. infestans* isolates collected during this project.

## **4.2 Materials and methods**

Detached leaflet tests were performed using a protocol adapted from Bakonyi and Cooke (2004) and are described below.

### **4.2.1 Timing and location**

The experiment ran from the 24<sup>th</sup> of March to the 4<sup>th</sup> of April 2014. Plants used to supply leaf material were grown during the winter in a heated and lit greenhouse at Henfaes Research Centre, Abergwynnregyn, UK (53° 14' N, 4° 01' W). Tomato cultivars with known *Ph-1*, *Ph-2* and *Ph-3* genotypes were chosen to provide a range of combinations of these genes (**Table 4.1**).

**Table 4.1: Plant Material Used**

Tomato cultivars with different *Ph*- gene combinations used to provide detached leaves for testing their response to a range of *P. infestans* isolates. Reference indicates the source of the genotype information. Ailsa Craig is a traditional variety with no known *P. infestans* resistance.

Cultivar Name	<i>Ph</i> -1	<i>Ph</i> -2	<i>Ph</i> -3	Reference
Ailsa Craig	+/+	+/+	+/+	Trad.
New Yorker	<i>Ph</i> -1/ <i>Ph</i> -1	+/+	+/+	(Robinson <i>et al.</i> 1967)
West Virginia 63	+/+	<i>Ph</i> -2/ <i>Ph</i> -2	+/+	(Gallegly 1964)
Legend	+/+	<i>Ph</i> -2/ <i>Ph</i> -2	+/+	(Savonen <i>et al.</i> 2008)
Plum Regal	+/+	+/+	<i>Ph</i> -3/+	(Gardner and Panthee 2010a)
Mountain Magic	+/+	<i>Ph</i> -2/+	<i>Ph</i> -3/+	(Gardner 2008)
NC2-CELBR	+/+	<i>Ph</i> -2/ <i>Ph</i> -2	<i>Ph</i> -3/ <i>Ph</i> -3	(Gardner and Panthee 2008)

Ailsa Craig is a traditional open pollinated cultivar which was bred in the very early 20<sup>th</sup> century (Stocks 2009), before any of the *Ph*- blight resistance genes had been introgressed into domesticated tomato, and it is highly susceptible to *P. infestans*. New Yorker is an OP cultivar which was introduced in the 1960s (Robinson *et al.* 1967) and carries homozygous *Ph*-1 (Hansen *et al.* 2014). West Virginia 63 is an OP cultivar which was also released in the 1960s (Gallegly 1964) and carries homozygous *Ph*-2 (Kim and Mutschler 2006). Likewise, Legend was released by Oregon State University in 2008 (Savonen *et al.* 2008), and is also an OP cultivar carrying homozygous *Ph*-2 (Hansen *et al.* 2014).

**Table 4.2: *P. infestans* isolates used in this study.**

“Isolate ID” is an arbitrary name assigned to each isolate collected in a survey carried out in 2013 (see **Chapter 2**), when the isolations were made from infected tomato plants. Clonal lineage of each isolate was determined using 12 SSR markers. The 8\_A1 isolate P23 was collected from potato; other isolates were collected from tomato.

Isolate ID	Origin	Clonal Lineage
T40	Abingdon, Oxfordshire	13_A2
T44	Guildford, Surrey	6_A1
P23	Bangor, Gwynedd	8_A1
T20	Leicester, Leicestershire	Unique

#### 4.2.2 Plant growing conditions

Seed of the tomato cultivars listed in **Table 4.1** was sown in trays of John Innes (JI) No. 2 compost (William Sinclair Holdings plc, Lincoln, UK) on the 20<sup>th</sup> of December 2013. Seedlings were pricked out and transplanted to 10 cm square pots of JI No. 2 compost on the 17<sup>th</sup> of January 2014, and subsequently repotted into 7-litre pots of JI No. 3 compost (William Sinclair Holdings plc, Lincoln, UK) on the 16<sup>th</sup> of February 2014. The compost was kept moist and plants were fed weekly with Miracle Gro soluble plant food (Scotts Miracle-Gro, Marysville, Ohio, USA) diluted according to the manufacturer’s instructions. Stems were supported with canes as required and plants were

not pruned. Plants were grown in a greenhouse at  $22 \pm 5$  °C daytime temperature, 7 °C minimum night temperature, with natural daylight supplemented with sodium growth lighting to give 16 hours of light.

Young, fully expanded, whole leaves were snapped from the stem at the axil. Immature leaves or those showing signs of senescence were not used. Leaves usually bore six axial leaflets, one terminal leaflet, and numerous smaller leaflets protruding from the rachis. Only axial leaflets were used for inoculation tests, and were removed from the rachis in the laboratory. Any leaflet with physical damage, deformity or visible disease was rejected where possible (although in some cases it was impossible to find sufficient leaflets without tolerating some infection by powdery mildew (*Oidium neolycopersici* or *Leveillula taurica*)).

### 4.2.3 Moist chambers for incubation of leaflets

Moist chambers for incubation of leaflets consisted of translucent plastic food containers with a slab of damp Oasis florist's foam (Smithers-Oasis UK Ltd., Tyne and Wear, UK) in the bottom. The plastic boxes measured 140 by 200 mm at the base by 100 mm deep and the florist's foam slabs measured 120 mm by 180 mm by 18 mm thick. Transmission of photosynthetically active radiation (PAR) through the lids of the boxes was measured using an AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, Washington, USA) and found to be approximately 80% of incident PAR (data not shown) and was found during an informal pilot study to be sufficient to prevent leaflet yellowing and visible senescence.

The boxes were placed on benches in a large greenhouse. The greenhouse was automatically ventilated during the day to maintain a temperature of 13 to 20 °C, and heated to maintain a minimum night temperature of 7 °C. To avoid direct sunlight overheating the moist chambers, the greenhouse roof was shaded with reflective shade netting and the walls were shaded with horticultural fleece (Henry Alty Ltd., Hesketh Bank, UK).

### 4.2.4 Inoculum preparation

#### 4.2.4.1 Selection of *P. infestans* isolates

Twenty-five *P. infestans* isolates collected in 2013 were assessed for aggressiveness by inoculating onto susceptible tomato cv. Ailsa Craig leaflets and measuring lesion diameter after six days (**Appendix 8.2**). Four isolates were selected due to high aggressive levels: aggressive isolates of 13\_A2, 6\_A1 and 8\_A1 genotype, and one "Unique" isolate (not from a known clonal lineage, henceforth "*Unique*"), which was the most aggressive isolate in the collection (**Table 4.2** Table 4.2). The only available 8\_A1 isolate was collected from potato, but in other cases isolates were collected from tomato plants.

#### 4.2.4.2 *Isolate restoration and transfer*

*P. infestans* isolates collected in summer 2013 were used to prepare inoculum. As the isolates had been cultured on artificial media for several months, which can reduce aggressiveness on plant tissue, they were initially passaged through susceptible cv. Ailsa Craig tomato leaves for several generations to restore aggressiveness (Jinks and Grindle 1963). Initial transfer from agar to leaf was achieved by cutting several wedges of agar from the edge of the colony and placing them in the centre of tomato leaflets (abaxial side up) in a moist chamber, and incubating in diffuse natural light at moderate temperature (between 13 °C and 20 °C). Once a sporulating lesion had developed (after approximately seven days), sporangia were transferred to a new tomato leaflet by flooding the lesion with approximately 0.25 mL of Volvic mineral water (Danone Waters, Trowbridge, UK), agitating with a pipette tip, and pipetting 15 µL of the resulting sporangia suspension onto the abaxial side of a new leaflet, at a location immediately to the left or right of the midrib and approximately centrally located along it. Volvic mineral water was used as it has been found to give higher infection success frequencies than pure deionised or distilled water (David Shaw, Sarvari Research Trust, Bangor, *pers. comm.*). The newly inoculated leaflet was then incubated as described previously, and the isolate transfer repeated once sporulation was apparent. This procedure was repeated for a minimum of two further cycles (to achieve a minimum of four generations on tomato leaflets) before the inoculum was used in experiments.

#### 4.2.4.3 *Preparation of sporangial suspension*

To prepare a sporangial suspension for experimental inoculations, one or more lesions were washed in approximately 10 mL of Volvic mineral water, agitating to dislodge sporangia. The concentration of the resulting sporangial suspension was determined by pipetting 4 µL of suspension onto a glass slide as a two-to-four-centimetre long streak, and counting sporangia manually by surveying the streak end to end under a Novex 65x zoom binocular microscope (Euromex Microscopen BV, Arnhem, The Netherlands). This method was found to be quicker than, and at least as consistent as, manual counting using a haemocytometer (data not shown). Three replicate counts were made and averaged. The suspension was diluted with Volvic mineral water to achieve a final concentration of 10,000 sporangia mL<sup>-1</sup>. The suspension was then chilled at 4 °C for one to three hours to stimulate zoospore release. When zoospores were visible, the suspension was used for experimental inoculations, agitating it by swirling throughout the experimental setup to maintain a homogeneous zoosporal/sporangial concentration.

#### 4.2.5 **Experiment setup**

Six leaflets of a single tomato cultivar were placed in a moist chamber and inoculated with a single *P. infestans* strain (thus each chamber contained six replicates of the same treatment). The



inoculation procedure was as described under *Isolate transfer*, although a 10,000 sporangia mL<sup>-1</sup> suspension (as described under *Inoculum preparation*) was used. Seven replicate moist chambers of each host cultivar / pathogen isolate combination were made. The seven replicate chambers were arranged on the greenhouse benchtop in a non-random, stratified layout (**Figure 4.1**).

Inoculation took place on the 24<sup>th</sup> of March 2014 and the experiment ran for 11 days. Leaflets were checked daily (in the morning) for signs of active infection from the 27<sup>th</sup> of March (3 Days Post Inoculation). Assessment of lesion diameter took place on the 31<sup>st</sup> of March (7 DPI) and the 4<sup>th</sup> of April (11 DPI), when destructive assessment of sporulation intensity also took place. The assessments are described below.

#### 4.2.6 Data collected

Three parameters were directly measured: Infection Efficiency (i.e. proportion of inoculated leaflets which developed sporulating lesions), Lesion Diameter at a given time after inoculation, and Sporulation Intensity (no of sporangia produced by a given lesion area) at the end of the experiment.

##### 4.2.6.1 Infection Efficiency

Infection Efficiency (henceforth IE) was the proportion of inoculated leaflets which went on to develop an expanding and sporulating lesion (i.e. developed an active infection as opposed to a small, nonexpanding patch of necrotic tissue caused by a hypersensitive resistance response) during the course of the experiment was calculated by dividing the number of leaflets which became actively infected by the total number of leaflets. In some instances, the total was fewer than the six leaflets at the start of the experiment owing to leaflet loss (see **4.2.7 Leaflet Loss**).

##### 4.2.6.2 Lesion Diameter

Lesion Diameter (LD) was the approximate mean diameter of a lesion after a given time interval. Mean diameter of all lesions was estimated at 7 and 11 DPI by comparison with a sheet of reference images (**Appendix 8.1**). Where lesions were non-circular, the diameter was assigned by estimating which circle best matched the lesion in terms of area.

##### 4.2.6.3 Mean Sporulation Intensity

At 11 DPI a 16 mm diameter circular disc was punched from the centre of largest replicate lesion in each box. The disc was placed in a 20 mL glass sample vial and a known quantity of fixative solution was added. Fixative solution prevented sporangia from releasing zoospores, and consisted of an aqueous solution of copper sulphate (0.04 M) and sodium acetate (0.2 M) adjusted to pH 5.4 using acetic acid (Legard *et al.* 1995). The quantity (2, 4 or 6 mL) was selected according

to the observed density of sporangia growing from the lesion, to achieve a sporangia concentration of <100 sporangia per 4  $\mu$ L of suspension to facilitate counting. The vial was shaken vigorously for approximately five seconds to dislodge a maximal number of sporangia. (Pilot experiments (*data not shown*) showed that the number of sporangia dislodged into the solution depended on time and force of shaking, but plateaued after five seconds of vigorous shaking). The number of sporangia in 4  $\mu$ L of suspension was counted as described in **Inoculum preparation (4.2.4)**. If it was apparent that the concentration of sporangia was substantially greater than 100 sporangia per 4  $\mu$ L when the first sample was taken, additional fixative solution was added to dilute the sporangia suspension to a concentration of <100 sporangia per 4  $\mu$ L. Once a satisfactory dilution of sporangia suspension had been achieved, three replicate counts of the sporangia in 4  $\mu$ L samples of suspension were made. Finally, the counts for each tube were corrected as follows:

$$\text{Corrected count} = \text{Observed count} \times \text{Volume of fixative solution}$$

#### 4.2.7 Leaflet loss

Some leaflets were lost during the course of the experiment due to infection with saprotrophic/necrotrophic organisms other than *P. infestans*, such as *Botrytis cinerea*. Leaflets lost prior to seven days post inoculation were excluded from all analyses. Leaflets lost later in the experiment, (seven days or more post inoculation) were included in the study of *Infection efficiency*. These leaflets were counted as uninfected if they had not already developed an active *P. infestans* infection before being lost, or counted as infected if they did develop an active *P. infestans* infection prior to being lost. If infected leaflets remained in otherwise good condition at the time of measuring the first of two *Lesion size* measurements, this measurement was made, although by definition, any leaflets that were lost during the experiment were no longer in good condition at the time of assessing final *Lesion size* or *Sporulation intensity* at the end of the experiment.

#### 4.2.8 Data analysis

##### 4.2.8.1 Comparison of Infection Efficiency

To test for a significant effect of cultivar on Infection Efficiency, Pearson Chi-squared tests were used to compare the ratio of infected vs uninfected leaflets across the seven tomato cultivars. Data from the four *P. infestans* isolates was analysed separately. Bonferroni-corrected Z-tests were used to identify significant differences between tomato cultivars. All tests were performed in R (R Core Team 2014) using the *stats* package.

#### 4.2.8.2 *Comparison of Mean Lesion Size*

To test for a significant effect of tomato genotype on lesion size, Kruskal-Wallis tests were used. To perform post-hoc multiple comparisons, Benjamini-Hochberg adjusted Dunn Tests were used, allowing a false-detection-rate of 0.05. Datasets from the different pathogen isolates were analysed separately. All tests were performed in R (R Core Team 2014) using the package *Dunn.test* (Dinno 2014). The resulting p-values were then used to create letter-coded groupings with the R package *multcompView* (Graves *et al.* 2012).

#### 4.2.8.3 *Comparison of Sporulation Intensity*

To test for a significant effect of tomato genotype on Sporulation Intensity, the same tests and parameters were used as for lesion size.

Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 N. York
13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 N. York
6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	
8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique Ailsa	Unique Plum R.	Unique Legend	
Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	
13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	
6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique Ailsa	Unique Plum R.	Unique Legend	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York
8_A1 Ailsa	8_A1 Plum R.	8_A1 Legend	8_A1 NC2-C	8_A1 WV-63	8_A1 M. Mag.	8_A1 N. York	6_A1 Ailsa	6_A1 Plum R.	6_A1 Legend	6_A1 NC2-C	6_A1 WV-63	6_A1 M. Mag.	6_A1 N. York	13_A2 Ailsa	13_A2 Plum R.	13_A2 Legend	13_A2 NC2-C	13_A2 WV-63	13_A2 M. Mag.	13_A2 N. York	Unique NC2-C	Unique WV-63	Unique M. Mag.	Unique N. York

Figure 4.1: Layout of moist chambers.

Layout of 49 boxes of leaflets on greenhouse bench for all detached leaflet inoculation tests. Colour indicates *P. infestans* isolate (also indicated in the first line of text in each box). Tomato cultivar is indicated in the second line of text in each box.

## 4.3 Results

### 4.3.1 Infection Efficiency

#### 4.3.1.1 Ailsa Craig and New Yorker

Differences in IE between cultivars were seen at 6 DPI. Infection of Ailsa Craig, the susceptible control cultivar, was over 95% when inoculated with the 13\_A2, 8\_A1 and *Unique* isolates, and slightly lower (87.5%) with 6A1 (**Figure 4.2** and **Table 4.3**). Ailsa Craig was always significantly more susceptible than cultivars with resistance genes, except for New Yorker (*Ph-1*), which was similarly susceptible to Ailsa Craig when inoculated with 13\_A2 or 6\_A1, although slightly but significantly less so when inoculated with 8\_A1 or *Unique*. No additional infections developed on either Ailsa Craig or New Yorker between 6 and 11 DPI (**Figure 4.3** and **Table 4.4**).

#### 4.3.1.2 Plum Regal

IE on Plum Regal (heterozygous *Ph-3*) did not differ greatly among *P. infestans* isolates, ranging from 57.1% with 6\_A1 to 73.1% with 13\_A2 and 8\_A1 at both 6 and 11 DPI; no additional infection developed on Plum Regal after 6 DPI. With most isolates, Plum Regal was slightly less resistant than either Legend or West Virginia 63 (both homozygous *Ph-2*), although this difference was only statistically significant with isolates 8\_A1 (71.4% for Plum Regal compared with 21.4% and 14.3% for Legend and West Virginia 63 respectively at 6 DPI, and 78.0% for Legend at 11DPI) and 13\_A2 (73.8% on Plum Regal and 35.7% on Legend at 6 DPI). Plum Regal showed slightly but significantly lower IE than Ailsa Craig with all *P. infestans* isolates.

#### 4.3.1.3 Legend and West Virginia 63

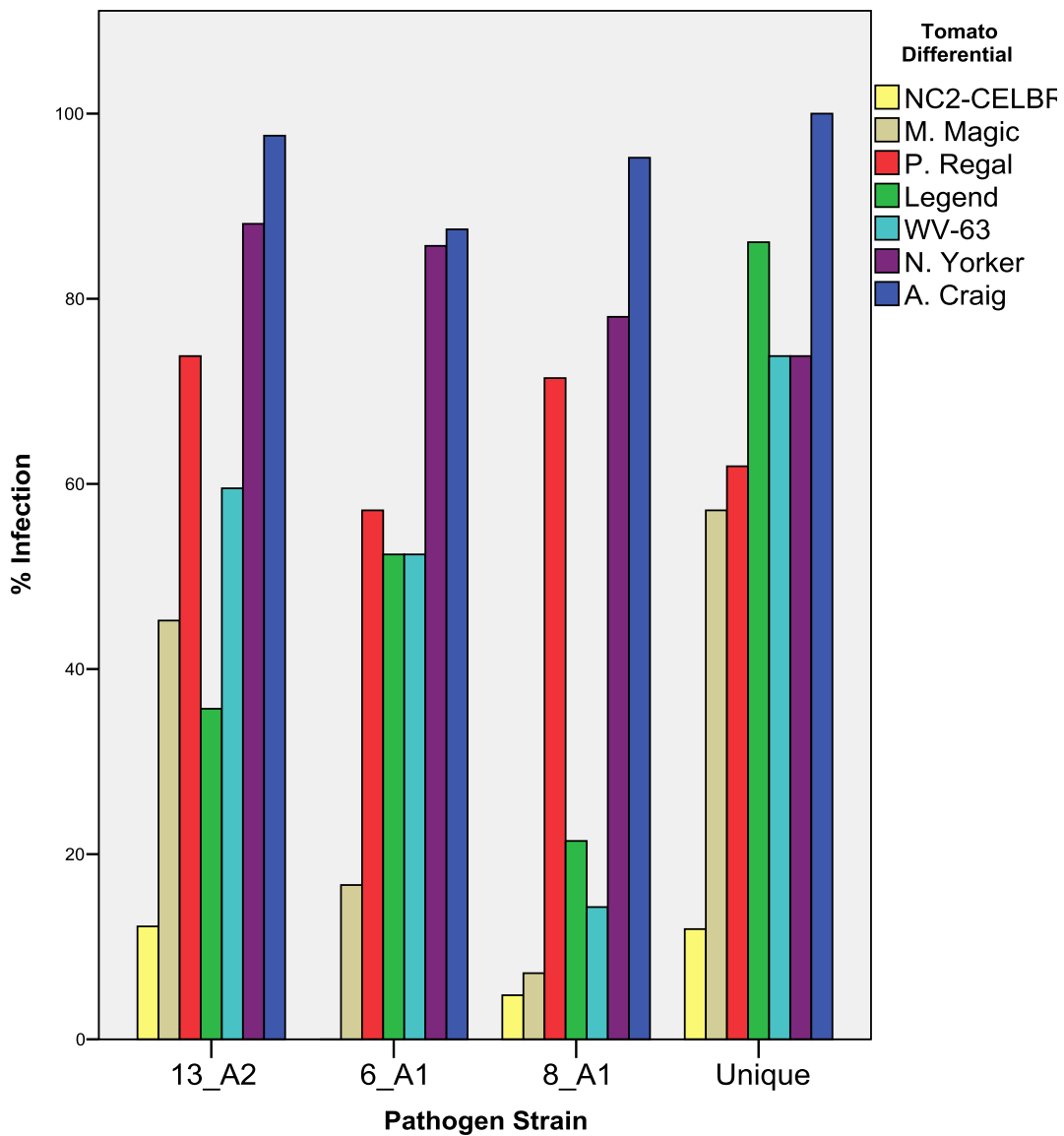
IE differences among *P. infestans* isolates were greater on the two homozygous *Ph-2* cultivars Legend and West Virginia 63 than on other cultivars. High IE by *Unique* occurred on these cultivars, and was only slightly (although significantly) less than on Ailsa Craig (73.8% and 86.1% on West Virginia 63 and Legend respectively, compared with 100% on Ailsa Craig). In contrast, IE by 8\_A1 was low at 6DPI (14.3% and 21.4% on West Virginia 63 and Legend respectively) and not significantly different from IE on the most resistant cultivars NC2-CELBR and Mountain Magic. IE on the *Ph-2* homozygotes by 13\_A2 and 6\_A1 was intermediate.

The IE of 13\_A2 on the homozygous *Ph-2* cultivars increased from moderate levels at 6 DPI to 70-80% by 11 DPI. IE by 8\_A1 showed a similar pattern on West Virginia 63, although Legend only increased to 38.1% at 11 DPI, making it the least severely infected cultivar when inoculated with 8\_A1.

#### 4.3.1.4 NC2-CELBR and Mountain Magic

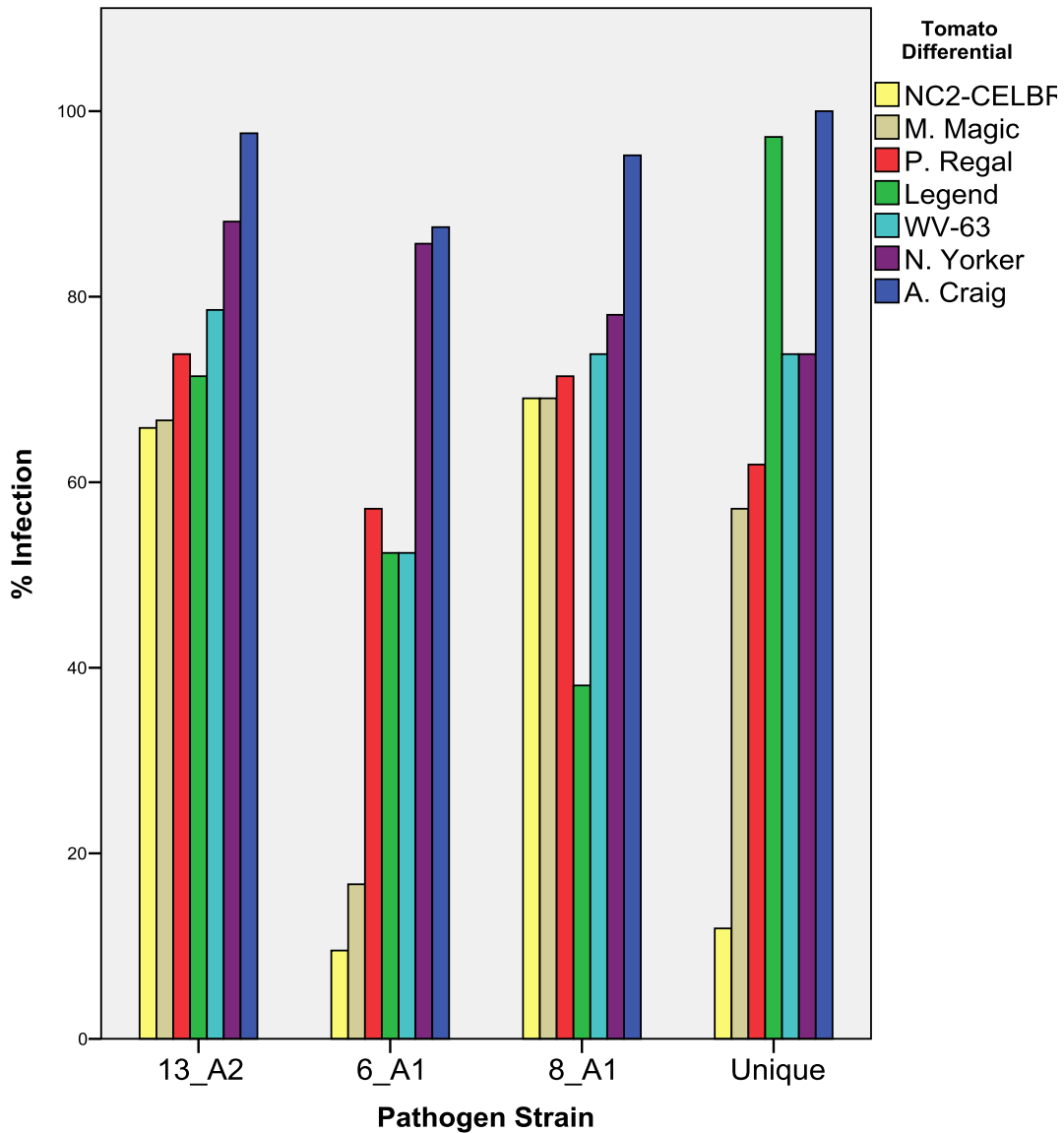
Homozygous *Ph-2* and *Ph-3* in combination (in NC2-CELBR) gave strong resistance to all isolate. NC2-CELBR was consistently the least susceptible cultivar, with infection frequencies between 0% and 11.9% at 6 DPI. When *Ph-2* and *Ph-3* were combined in the heterozygous state (in Mountain Magic) the resistance against 6\_A1 and 8\_A1 was also strong (16.7% and 7.1% IE respectively), although resistance to infection by 13\_A2 and *Unique* was lower (45.2% and 57.1% respectively).

Large increases (up to 64.3 percentage points) occurred in the IE on NC2-CELBR and Mountain Magic inoculated with 13\_A2 and 8\_A1 IE between the 6 and 11 DPI, increasing their IE levels to a similar level to other cultivars, with only Ailsa Craig showing significantly greater susceptibility.



**Figure 4.2: Bar plot of Infection efficiency (IE) at 6 DPI**

Percentage of inoculated tomato leaflets which became infected by 6 DPI following inoculation with different *P. infestans* strains. No. of replicate leaflets for each treatment was 42 in all but the following cases: 13\_A2 on NC2-CELBR (41); 6\_A1 on A. Craig (40); 8\_A1 on N. Yorker (41); *Unique* on Legend (36).



**Figure 4.3: Bar plot of Infection efficiency (IE) at 11 DPI**

Percentage of inoculated tomato leaflets which became infected by 11DPI following inoculation with different *P. infestans* strains. No. of replicate leaflets for each treatment was 42 in all but the following cases: 13\_A2 on NC2-CELBR (41); 6\_A1 on A. Craig (40); 8\_A1 on N. Yorker (41); *Unique* on Legend (36).



**Table 4.3: infection efficiency of four *P. infestans* isolates on tomato genotypes at 6 days post inoculation.**

Unf. = absolute number of inoculated leaflets that did not become infected within 12 days. Inf. = absolute number of leaflets that did develop an actively expanding and/or sporulating lesion. %Inf = percentage of leaflets which developed an actively expanding and/or sporulating lesion. Group indicates results of Z-tests between cultivars; cultivars which share a letter do not differ significantly for infection efficiency, whilst those which do not share at least one letter do differ at the 0.05 level (Bonferroni-corrected).

Cultivar & Ph- genes	13_A2					Cultivar & Ph- genes	6_A1				
	Unf.	Inf.	% Inf.	N	Group		Unf.	Inf.	% Inf.	N	Group
NC2-CELBR -- 22 33	36	5	12.2	41	e	NC2-CELBR -- 22 33	42	0	0	42	d
M. Magic -- 2- 3-	23	19	45.2	42	d	M. Magic -- 2- 3-	35	7	16.7	42	c
P. Regal 33	11	31	73.8	42	bc	P. Regal 33	18	24	57.1	42	b
Legend -- 22 --	27	15	35.7	42	d	Legend -- 22 --	20	22	52.4	42	b
WV-63 -- 22 --	17	25	59.5	42	cd	WV-63 -- 22 --	20	22	52.4	42	b
N. Yorker 11 ---	5	37	88.1	42	ab	N. Yorker 11 ---	6	36	85.7	42	a
A. Craig ----	1	41	97.6	42	a	A. Craig ----	5	35	87.5	40	a
<b>Mean</b>	<b>120</b>	<b>173</b>	<b>59</b>	<b>293</b>		<b>Mean</b>	<b>146</b>	<b>146</b>	<b>50</b>	<b>292</b>	

Cultivar & Ph- genes	8_A1					Cultivar & Ph- genes	Unique				
	Unf.	Inf.	% Inf.	N	Group		Unf.	Inf.	% Inf.	N	Group
NC2-CELBR -- 22 33	40	2	4.8	42	c	NC2-CELBR -- 22 33	37	5	11.9	42	d
M. Magic -- 2- 3-	39	3	7.1	42	c	M. Magic -- 2- 3-	18	24	57.1	42	c
P. Regal 33	12	30	71.4	42	b	P. Regal 33	16	26	61.9	42	c
Legend -- 22 --	33	9	21.4	42	c	Legend -- 22 --	5	31	86.1	36	b
WV-63 -- 22 --	36	6	14.3	42	c	WV-63 -- 22 --	11	31	73.8	42	bc
N. Yorker 11 ---	9	32	78	41	b	N. Yorker 11 ---	11	31	73.8	42	bc
A. Craig ----	2	40	95.2	42	a	A. Craig ----	0	42	100	42	a
<b>Mean</b>	<b>171</b>	<b>122</b>	<b>41.6</b>	<b>293</b>		<b>Mean</b>	<b>98</b>	<b>190</b>	<b>66</b>	<b>288</b>	

13\_A2 Pearson Chi-Squared = 94.3, df = 6,  $p < 0.001$ ; 6\_A1 Pearson Chi-Squared = 105.6, df = 6,  $p < 0.001$ ; 8\_A1 Pearson Chi-Squared = 151.4, df = 6,  $p < 0.001$ ; Unique Pearson Chi-Squared = 86.3 df = 6  $p < 0.001$

**Table 4.4: infection efficiency of four *P. infestans* isolates on a range of tomato genotypes at 11 days post inoculation.**

Unf. = absolute number of inoculated leaflets that did not become infected within 12 days. Inf. = absolute number of leaflets that did develop an actively expanding and/or sporulating lesion. %Inf = percentage of leaflets which developed an actively expanding and/or sporulating lesion. Group indicates results of Z-tests between cultivars; cultivars which share a letter do not differ significantly for infection efficiency, whilst those which do not share at least one letter do differ at the 0.05 level (Bonferroni-corrected).

Cultivar and Ph- genes	13_A2					Cultivar and Ph- genes	6_A1				
	Unf.	Inf.	% Inf.	N	Group		Unf.	Inf.	% Inf.	N	Group
NC2-CELBR -- 22 33	14	27	65.9	41	b	NC2-CELBR -- 22 33	38	4	9.5	42	c
M. Magic -- 2- 3-	14	28	66.7	42	b	M. Magic -- 2- 3-	35	7	16.7	42	c
WV-63 -- 22 --	9	33	78.6	42	ab	P. Regal 33	18	24	57.1	42	b
P. Regal 33	11	31	73.8	42	b	Legend -- 22 --	20	22	52.4	42	b
Legend -- 22 --	12	30	71.4	42	b	WV-63 -- 22 --	20	22	52.4	42	b
N. Yorker 11 ---	5	37	88.1	42	ab	N. Yorker 11 ---	6	36	85.7	42	a
A. Craig -----	1	41	97.6	42	a	A. Craig -----	5	35	87.5	40	a
<b>Mean</b>	<b>66</b>	<b>227</b>	<b>77.5</b>	<b>293</b>		<b>Mean</b>	<b>142</b>	<b>150</b>	<b>51.4</b>	<b>292</b>	

Cultivar and Ph- genes	8_A1					Cultivar and Ph- genes	Unique				
	Unf.	Inf.	% Inf.	N	Group		Unf.	Inf.	% Inf.	N	Group
NC2-CELBR -- 22 33	13	29	69	42	b	NC2-CELBR -- 22 33	37	5	11.9	42	c
M. Magic -- 2- 3-	13	29	69	42	b	M. Magic -- 2- 3-	18	24	57.1	42	b
P. Regal 33	12	30	71.4	42	b	P. Regal 33	16	26	61.9	42	b
WV-63 -- 22 --	11	31	73.8	42	b	WV-63 -- 22 --	11	31	73.8	42	b
Legend -- 22 --	26	16	38.1	42	c	N. Yorker 11 ---	11	31	73.8	42	b
N. Yorker 11 ---	9	32	78	41	b	Legend -- 22 --	1	35	97.2	36	a
A. Craig -----	2	40	95.2	42	a	A. Craig -----	0	42	100	42	a
<b>Mean</b>	<b>86</b>	<b>207</b>	<b>70.6</b>	<b>293</b>		<b>Mean</b>	<b>94</b>	<b>194</b>	<b>67.4</b>	<b>288</b>	

13\_A2 Pearson Chi-Squared = 19.7, df = 6, p = 0.003; 6\_A1 Pearson Chi-Squared = 91.0, df = 6, p < 0.001; 8\_A1 Pearson Chi-Squared = 35.1, df = 6, p < 0.001; Unique Pearson Chi-Squared = 97.9, df = 6 p < 0.001

### 4.3.2 Mean lesion diameter

There were large differences in the mean LD (across all cultivars) caused by the four isolates between 6 and 11 DPI. The average lesion diameter across all cultivars of isolates 6\_A1, 8\_A1 and *Unique* almost doubled, with slower lesion growth (lower increase ratio) by the 13\_A2 isolate than other isolates (**Table 4.5**).

**Table 4.5: Mean lesion diameter produced by each isolate across all cultivars**

The mean lesion diameter produced by each isolate at 6 and 11 days post inoculation, and the ratio of the two values.

Isolate	6 DPI		11 DPI		Increase ratio from 6 to 11 DPI
	Mean LD (cm)	N	Mean LD (cm)	N	
13_A2	0.83	73	1.47	213	1.77
6_A1	1.15	146	2.01	189	1.75
8_A1	0.70	94	1.31	178	1.87
<i>Unique</i>	1.30	181	2.54	213	1.95

For most isolates, the increase in lesion size between 6 and 11 DPI was fairly uniform across cultivars, such that the assignment of cultivars into statistically similar ranking groups did not differ greatly between the two dates (**Table 4.6** and **Table 4.7**). There were notable exceptions to this rule, however (highlighted below).

#### 4.3.2.1 *Ailsa Craig and New Yorker*

At 6 DPI, all isolates produced their largest lesions on New Yorker (**Figure 4.4**). Lesions on *Ailsa Craig* were smaller than on New Yorker in all cases, although not significantly so (**Table 4.6**). Mean Lesion Diameter (LD) on these susceptible cultivars varied greatly with *P. infestans* isolate, with 6\_A1 and *Unique* producing lesions with 1.60-1.92 cm mean diameter, while 13\_A2 and 8\_A1 produced 0.81-1.09 cm diameter lesions.

By 11 DPI, mean LD on *Ailsa Craig* and New Yorker had increased in approximate proportion to the mean in the inoculations with 6\_A1, 8\_A1 and *Unique* (**Figure 4.5**). With 13\_A2, *Ailsa Craig* had increased in this manner, although there had been very little change in the mean diameter of lesions on New Yorker (which had increased from 1.09 cm at 6 DPI to 1.22 cm by 11 DPI).

#### 4.3.2.2 *Plum Regal*

*Plum Regal*, with heterozygous *Ph-3*, exhibited limited lesion growth at 6 DPI, with the mean diameter ranging from 0.63 cm (with 8\_A1) to 1.04 cm (with *Unique*). In all cases, *Plum Regal* was not significantly less resistant than the most resistant cultivars.

By 11 DPI, the mean diameter of lesions on Plum Regal (heterozygous *Ph-3*) caused by all isolates had almost doubled, as was the case with Ailsa Craig and New Yorker. The increase in mean LD was slightly larger when inoculated with 6\_A1, 8\_A1 and *Unique* (all more than 2×) compared with 13\_A2 (1.54×).

#### 4.3.2.3 *Legend and West Virginia 63*

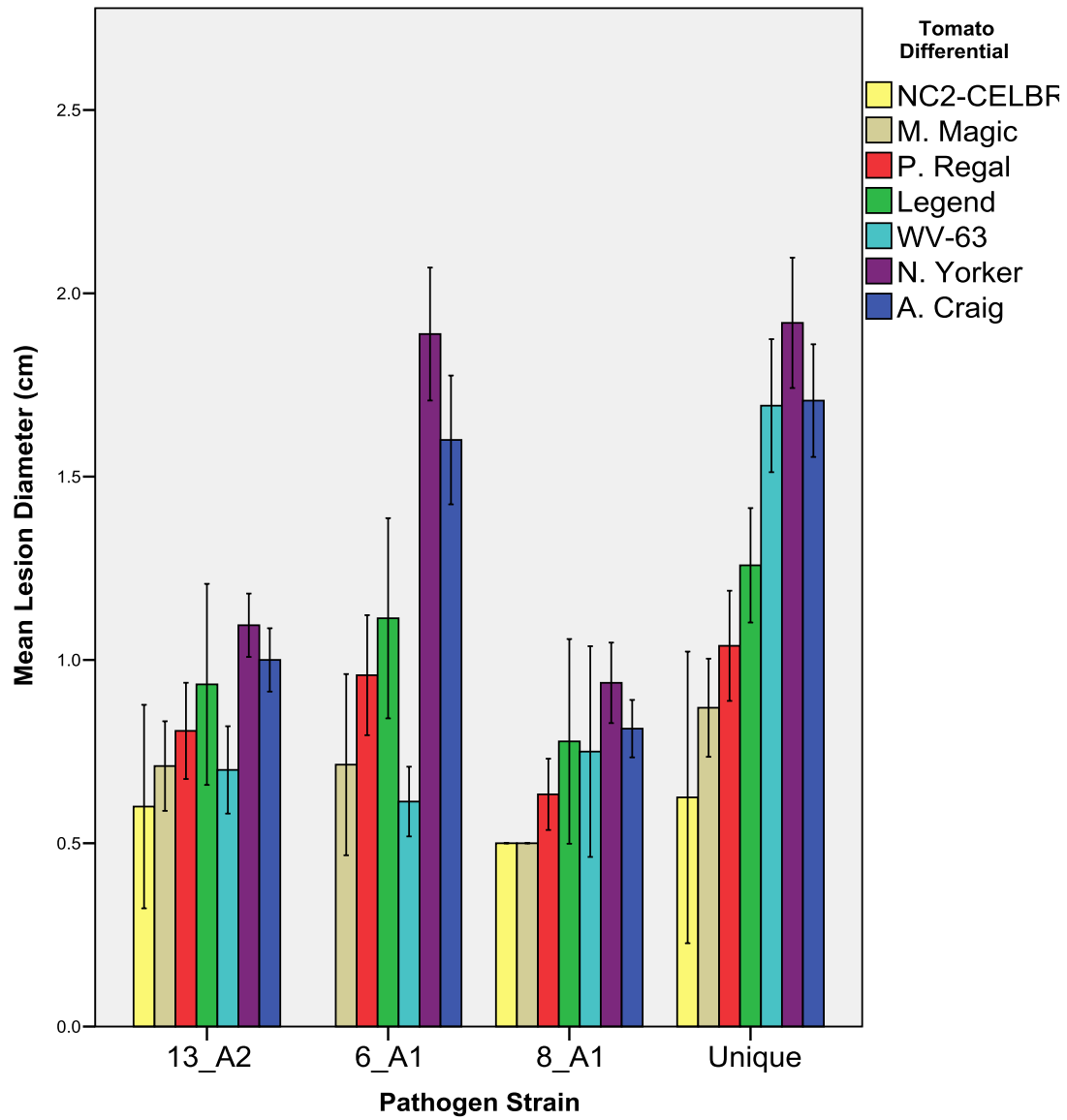
Legend and West Virginia 63 (both carrying homozygous *Ph-2*) showed similar responses with 13\_A2, 6\_A1, and 8\_A1, with LDs ranging from 0.61 to 0.75 cm on West Virginia 63, and 0.78 to 1.11 cm on Legend (no significant difference among the cultivars in any case). When inoculated with *Unique*, both cultivars suffered larger lesions (as did most cultivars with this aggressive isolate) although West Virginia 63 showed particularly large lesions, with a mean diameter of 1.69 cm, which was not significantly different to that of the Ailsa Craig or New Yorker.

The rate of increase of lesion size on the two homozygous *Ph-2* lines Legend and West Virginia 1963 between 6 and 11 DPI was similar to the mean increase in lesion size for each *P. infestans* isolate, and these two lines remained in broadly the same position in the ranking throughout the experiment.

#### 4.3.2.4 *NC2-CELBR and Mountain Magic*

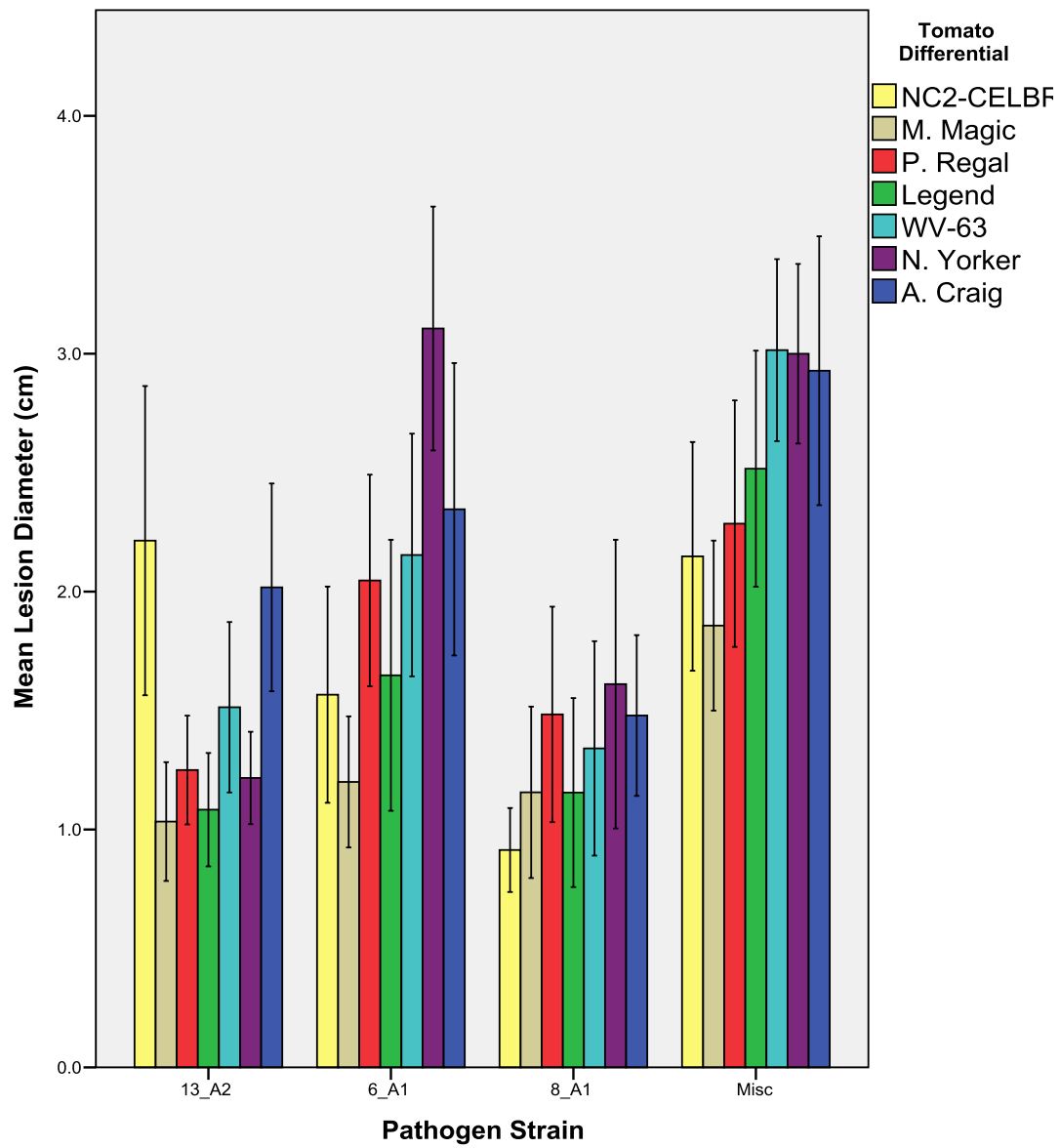
At 6 DPI, NC2-CELBR (homozygous *Ph-2* and *Ph-3*) and Mountain Magic (heterozygous for both genes) had developed the smallest lesions in most cases (**Figure 4.5**), with lesions ranging from 0.50 to 0.87 cm in diameter. Lesions on NC2-CELBR were always smaller than on Mountain Magic, although not significantly so.

The most striking departure from the average pattern of LD increase between 6 and 11 DPI was the lesion expansion on NC2-CELBR (homozygous *Ph-2* and *Ph-3*). For isolates 8\_A1 and *Unique*, the increase in mean LD was similar to the mean lesion expansion across all cultivars for these isolates. However, with isolate 13\_A2 there was a dramatic increase in mean LD on NC2-CELBR between 6 and 11 DPI. Mean diameter at 6 DPI was 0.6 cm, the smallest diameter amongst all cultivars. However, by 11 DPI it had risen to 2.21 cm, the largest value (not significantly different from mean LD on New Yorker or Ailsa Craig. In the case of 6\_A1, no leaflets had developed lesions at 6 DPI, so the apparently dramatic increase by 11 DPI (**Figure 4.5**) is in fact a product of there being no data at 6 DPI, and the mean LD at 11 DPI (1.57 cm) was second lowest after Mountain Magic. In contrast, lesion expansion on Mountain Magic followed a similar pattern to the overall means for most isolates (specifically, 1.45× with 13\_A2, 1.69× with 6\_A1, 2.32× with 8\_A1, and 2.14× with *Unique*).



**Figure 4.4: Mean lesion diameter (LD) at 6 DPI**

Mean lesion diameter in cm produced by four different *P. infestans* strains on leaflets of seven tomato cultivars at 6 DPI. Error bars represent 95% confidence intervals, with the exception of 8\_A1 on NC2-CELBR and M. Magic at 6 DPI, for which confidence intervals could not be calculated, as in both cases only two leaflets developed lesions that could be measured.



**Figure 4.5: Mean lesion diameter (LD) at 11 DPI**

Mean lesion diameter in cm produced by four different *P. infestans* strains on leaflets of seven tomato cultivars at 11 DPI. Error bars represent 95% confidence intervals, with the exception of 8\_A1 on NC2-CELBR and M. Magic at 6 DPI, for which confidence intervals could not be calculated, as in both cases only two leaflets developed lesions that could be measured.

**Table 4.6: Mean lesion diameter at 6 days post infection.**

Diameter is given in centimetres. Group indicates the result of Dunn Tests between cultivars, using Benjamini-Hochberg adjustment allowing a False Detection Rate (FDR) of 0.05. Cultivars which share a letter do not differ significantly for lesion size, whilst those which do not share at least one letter do differ significantly at FDR = 0.05. Rows are ordered by lesion size within each *P. infestans* isolate.

Cultivar & <i>Ph</i> - genes	13_A2			Cultivar & <i>Ph</i> - genes	6_A1		
	Diameter	N	Group		Diameter	N	Group
NC2-CELBR -- 22 33	0.6	5	a	NC2-CELBR -- 22 33		0	
M. Magic -- 2- 3-	0.71	19	a	M. Magic -- 2- 3-	0.71	7	ab
P. Regal 33	0.81	31	a	P. Regal 33	0.96	24	ab
Legend -- 22 --	0.93	15	ab	Legend -- 22 --	1.11	22	b
WV-63 -- 22 --	0.7	25	a	WV-63 -- 22 --	0.61	22	ab
N. Yorker 11 ---	1.09	37	b	N. Yorker 11 ---	1.89	36	c
A. Craig -----	1	41	b	A. Craig -----	1.6	35	c
<b>Mean</b>	<b>0.83</b>	<b>173</b>		<b>Mean</b>	<b>1.15</b>	<b>146</b>	

Cultivar & <i>Ph</i> - genes	8_A1			Cultivar & <i>Ph</i> - genes	Unique		
	Diameter	N	Group		Diameter	N	Group
NC2-CELBR -- 22 33	0.5	2	ab	NC2-CELBR -- 22 33	0.63	4	ab
M. Magic -- 2- 3-	0.5	2	ab	M. Magic -- 2- 3-	0.87	23	b
P. Regal 33	0.63	3	a	P. Regal 33	1.04	26	ab
Legend -- 22 --	0.78	9	ab	Legend -- 22 --	1.26	31	a
WV-63 -- 22 --	0.75	6	ab	WV-63 -- 22 --	1.69	31	c
N. Yorker 11 ---	0.94	32	b	N. Yorker 11 ---	1.92	31	c
A. Craig -----	0.81	40	b	A. Craig -----	1.71	35	c
<b>Mean</b>	<b>0.7</b>	<b>94</b>		<b>Mean</b>	<b>1.3</b>	<b>181</b>	

13\_A2 Kruskal-Wallis Chi-Squared = 39.6, df = 6, p < 0.001; 6\_A1 Kruskal-Wallis Chi-Squared = 75.5, df = 5, p < 0.001; 8\_A1 Kruskal-Wallis Chi-Squared = 22.1, df = 6, p < 0.001; *Unique* Kruskal-Wallis Chi-Squared = 83.1, df = 6 p < 0.001

**Table 4.7: Mean lesion diameter at 11 days post infection.**

Diameter is given in centimetres. Group indicates the result of Dunn Tests between cultivars, using Benjamini-Hochberg adjustment allowing a False Detection Rate (FDR) of 0.05. Cultivars which share a letter do not differ significantly for lesion size, whilst those which do not share at least one letter do differ significantly at FDR = 0.05. Rows are ordered by lesion size within each *P. infestans* isolate.

Cultivar & <i>Ph</i> - genes	13_A2			Cultivar & <i>Ph</i> - genes	6_A1		
	Diameter	N	Group		Diameter	N	Group
NC2-CELBR	2.21	27	bc	NC2-CELBR	1.57	15	ab
-- 22 33				-- 22 33			
M. Magic	1.03	28	a	M. Magic	1.2	30	a
-- 2- 3-				-- 2- 3-			
P. Regal	1.25	28	ab	P. Regal	2.05	32	ab
33				33			
Legend	1.08	30	a	Legend	1.65	27	ab
-- 22 --				-- 22 --			
WV-63	1.51	33	abc	WV-63	2.15	26	bc
-- 22 --				-- 22 --			
N. Yorker	1.22	32	ab	N. Yorker	3.11	33	c
11 -- --				11 -- --			
A. Craig	2.02	35	c	A. Craig	2.35	26	bc
-----				-----			
<b>Mean</b>	<b>1.47</b>	<b>213</b>		<b>Mean</b>	<b>2.01</b>	<b>189</b>	

Cultivar & <i>Ph</i> - genes	8_A1			Cultivar & <i>Ph</i> - genes	Unique		
	Diameter	N	Group		Diameter	N	Group
NC2-CELBR	0.91	29	a	NC2-CELBR	2.15	27	ab
-- 22 33				-- 22 33			
M. Magic	1.16	29	a	M. Magic	1.86	28	a
-- 2- 3-				-- 2- 3-			
P. Regal	1.48	27	a	P. Regal	2.29	28	abc
33				33			
Legend	1.16	16	a	Legend	2.52	30	abc
-- 22 --				-- 22 --			
WV-63	1.34	31	a	WV-63	3.02	33	c
-- 22 --				-- 22 --			
N. Yorker	1.61	24	a	N. Yorker	3	32	c
11 -- --				11 -- --			
A. Craig	1.48	22	a	A. Craig	2.93	35	bc
-----				-----			
<b>Mean</b>	<b>1.31</b>	<b>178</b>		<b>Mean</b>	<b>2.54</b>	<b>213</b>	

13\_A2 Kruskal-Wallis Chi-Squared = 25.7, df = 6,  $p < 0.001$ ; 6\_A1 Kruskal-Wallis Chi-Squared = 30.3, df = 6,  $p < 0.001$ ; 8\_A1 Kruskal-Wallis Chi-Squared = 11.8, df = 6,  $p = 0.07$ ; *Unique* Kruskal-Wallis Chi-Squared = 23.4, df = 6  $p < 0.001$



### 4.3.3 Sporulation intensity

Sporulation Intensity (SI) at 11 DPI was the trait most strongly dependant on *P. infestans* isolate, with a nearly 8-fold difference in the average SI of *Unique* (19.4 sporangia  $\mu\text{L}^{-1}$ ) compared with 8\_A1 (2.5 sporangia  $\mu\text{L}^{-1}$ ), and 13\_A2 and 6\_A1 intermediate between *Unique* and 8\_A1 (**Figure 4.6**). It was also the trait which exhibited the greatest variation across host cultivars, ranging from 0.7 sporangia  $\mu\text{L}^{-1}$  on NC2-CELBR to 34.6 sporangia  $\mu\text{L}^{-1}$  on New Yorker (an almost 50-fold difference) when these cultivars were inoculated with *Unique*, and large ranges with all other isolates also. However, this trait was also highly variable within treatments, leading to wide confidence intervals (**Figure 4.6**).

#### 4.3.3.1 *Ailsa Craig and New Yorker*

New Yorker and Ailsa Craig exhibited similar reactions to each other whichever isolate they were inoculated with. With 6\_A1, these cultivars exhibited particularly high sporulation compared to all of the other isolates.

#### 4.3.3.2 *Plum Regal, Legend, and West Virginia 63*

The responses of the two homozygous *Ph-2* cultivars Legend and West Virginia 63 were statistically similar with all isolates, and also statistically similar to that of Plum Regal (heterozygous *Ph-3*) with all isolates except for *Unique*, where Plum Regal was statistically less susceptible than West Virginia 63 (although not Legend).

#### 4.3.3.3 *Mountain Magic and NC2-CELBR*

Sporulation intensity was always low on Mountain Magic (heterozygous *Ph-2* and *Ph-3*) and NC2-CELBR exhibited little or no sporulation on most leaflets which did develop lesions, and correspondingly low values of mean SI. However, the most aggressive isolate (*Unique*) did produce a mean SI of 7.6 sporangia  $\mu\text{L}^{-1}$  on Mountain Magic, which was higher than the mean SI on even the most susceptible cultivars when they were inoculated with the less aggressive 8\_A1 isolate.

**Table 4.8: Mean sporulation intensity at 11 days post infection.**

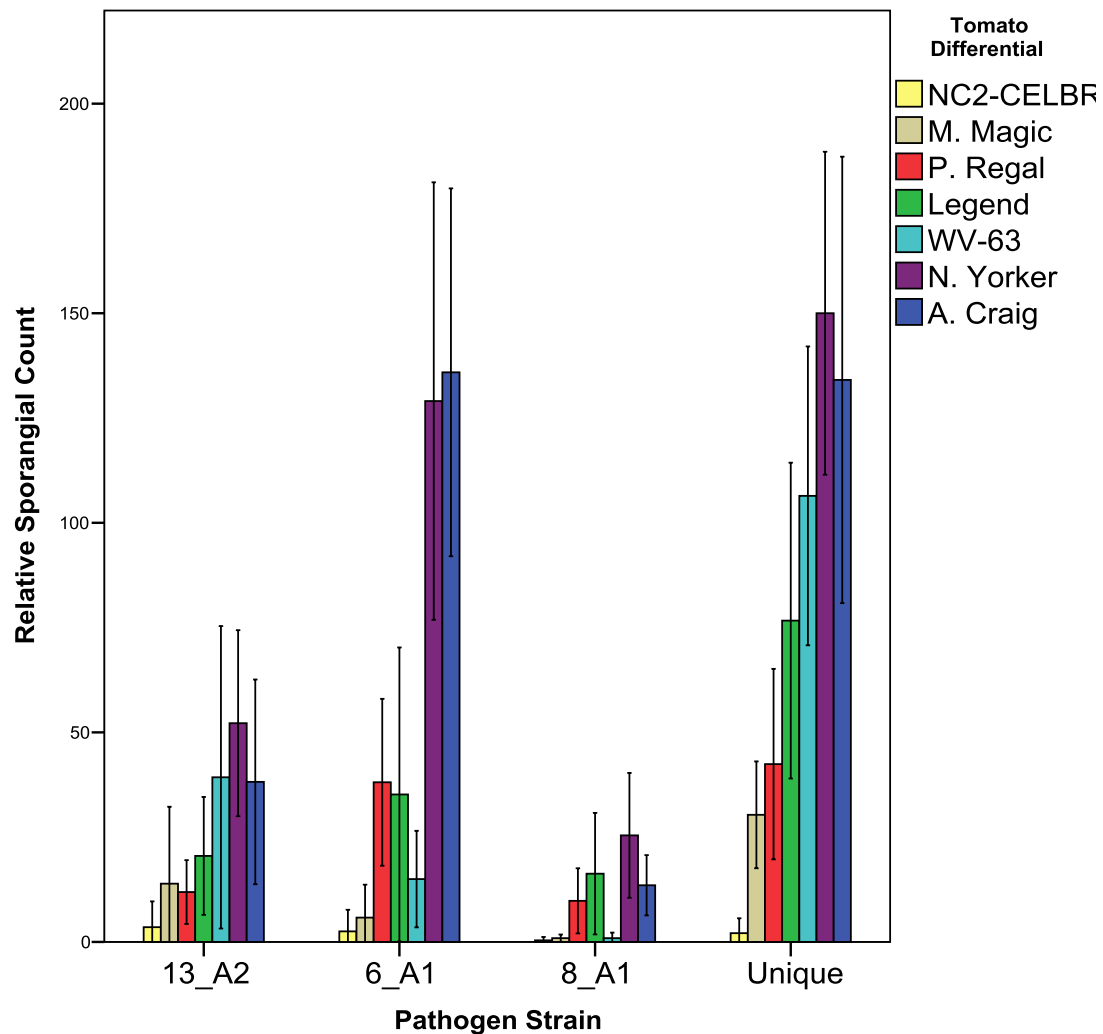
Diameter is given in centimetres. Group indicates the result of Dunn Tests between cultivars, using Benjamini-Hochberg adjustment allowing a False Detection Rate (FDR) of 0.05. Cultivars which share a letter do not differ significantly for lesion size, whilst those which do not share at least one letter do differ significantly at FDR = 0.05. Rows are ordered by lesion size within each *P. infestans* isolate. Spore Count is the mean number of sporangia  $\mu\text{l}^{-1}$  of sporangia suspension washed from a 16 mm diameter leaf disc in 2 ml of fixative solution.

Cultivar & <i>Ph</i> - genes	13_A2			Cultivar & <i>Ph</i> - genes	6_A1		
	Spore Count	N	Group		Spore Count	N	Group
NC2-CELBR -- 22 33	1.2	5	A	NC2-CELBR -- 22 33	1.1	4	a
M. Magic -- 2- 3-	3.5	7	Ab	M. Magic -- 2- 3-	1.5	7	a
P. Regal 33	3	7	Ab	P. Regal 33	9.5	7	ab
Legend -- 22 --	5.1	7	abc	Legend -- 22 --	8.8	7	a
WV-63 -- 22 --	11.5	6	bc	WV-63 -- 22 --	3.8	7	a
N. York 11 ---	13	7	C	N. York 11 ---	32.2	7	b
A. Craig -----	11.2	6	C	A. Craig -----	34	7	b
<b>Mean</b>	<b>6.9</b>	<b>45</b>		<b>Mean</b>	<b>13</b>	<b>46</b>	

Cultivar & <i>Ph</i> - genes	8_A1			Cultivar & <i>Ph</i> - genes	Unique		
	Spore Count	N	Group		Spore Count	N	Group
NC2-CELBR -- 22 33	0.2	4	a	NC2-CELBR -- 22 33	0.7	5	a
M. Magic -- 2- 3-	0.3	6	ab	M. Magic -- 2- 3-	7.6	7	ab
P. Regal 33	2.5	7	abc	P. Regal 33	10.6	7	ab
Legend -- 22 --	4.1	7	bc	Legend -- 22 --	22.4	6	bc
WV-63 -- 22 --	0.3	5	ab	WV-63 -- 22 --	26.6	7	c
N. York 11 ---	6.4	7	c	N. York 11 ---	34.6	7	c
A. Craig -----	4	6	c	A. Craig -----	33.6	7	c
<b>Mean</b>	<b>2.5</b>	<b>42</b>		<b>Mean</b>	<b>19.4</b>	<b>46</b>	

13\_A2 Kruskal-Wallis chi-squared = 14.8, df = 6, p-value = 0.02; 6\_A1 Kruskal-Wallis chi-squared = 29.0, df = 6, p-value = 0; 8\_A1 Kruskal-Wallis chi-squared = 22.6, df = 6, p-value = 0; Unique Kruskal-Wallis chi-squared = 31.7, df = 6, p-value = 0.



**Figure 4.6: Sporulation intensity**

Sporulation intensity measured as no. of sporangia per  $\mu\text{l}$  of suspension resulting from washing a 16 mm diameter circular disc cut from the centre of each lesion in 2 mL of fixative solution. In most cases, independent replicate counts were made from seven different leaf discs, although for the following treatments fewer lesions were available: 13\_A2 x NC2-CELBR (5), A. Craig (6), WV-63 (6); 6\_A1 x NC2-CELBR (4); 8\_A1 x NC2-CELBR (4), M. Magic (6); WV-63 (5), A. Craig (6); *Unique* x NC2-CELBR (5), Legend (6). Error bars indicate 95% confidence intervals.

## 4.4 Discussion

### 4.4.1 Overall performance of differential cultivars

#### 4.4.1.1 Ailsa Craig and New Yorker

*Ph-1* appeared to produce a small reduction in infection efficiency, although had no effect on other disease parameters. High frequencies of infection were observed on Ailsa Craig and New Yorker. Frequencies close to 100% were expected for Ailsa Craig, which carries no known resistance genes, and also for New Yorker, as *Ph-1* is generally considered to offer no resistance to contemporary *P. infestans* isolates (Foolad *et al.* 2008; Nowakowska *et al.* 2014; Nowicki *et al.* 2013). However, the small reduction in IE exhibited by New Yorker over Ailsa Craig for all isolates (although only statistically significant in the case of 6A\_1 and *Unique*) may be an example of the residual effect of “defeated” R-genes reported in potato by Rauscher *et al.* (2008), Stewart *et al.* (2003), and Tan *et al.* (2008). However, when mean LD and SI were considered, New Yorker generally exhibited statistically similar (although often greater) symptom development than Ailsa Craig, although with the 13\_A2 isolate, the expansion of lesions between 6 and 11 DPI was slightly depressed compared to Ailsa Craig, but this did not result in a statistically significant change in the ranking of the two isolates.

It should be remembered that Ailsa Craig and New Yorker are likely to have quite different genetic backgrounds in addition to their different *Ph*- genotype, and therefore the higher SI and LD seen on New Yorker is almost certainly due to this fact. For example, thicker leaf tissue with a greater nutrient content could quite plausibly cause more intense sporulation.

#### 4.4.1.2 Plum Regal

Heterozygous *Ph-3* in Plum Regal appeared to have only a modest and not always statistically significant effect on IE. Plum Regal showed moderate mean LD at both 6 and 11 DPI, which was always significantly less than that on Ailsa Craig and New Yorker at 6 DPI, although had increased to the point where this was not always so at 11 DPI. Plum Regal also showed a moderately reduced SI compared with Ailsa Craig. Overall, the moderate reduction in SI and LD could lead to a useful reduction in inoculum pressure in an infected field. The fairly consistent resistance to all isolates tested (in line with findings by Black *et al.* (1996a) and Nowicki *et al.* (2013)) would certainly be valuable in a British field situation where plants would potentially be exposed to a diverse population of *P. infestans* genotypes.

The performance of tomato cultivars in the field trials described in **Chapter 2** is difficult to compare directly with the results presented here, as only a single line in 2013 (cultivar Jasper) carried heterozygous *Ph-3* alone. Jasper experienced high levels of fruit and foliage disease

statistically similar to those seen on susceptible lines, although lines with homozygous *Ph-3* showed medium to large reductions in fruit and foliage disease compared to susceptible lines. The particularly strong effect of *Ph-3* genotype on fruit disease seen in the 2012 and 2013 field trials may well come primarily from the effect of *Ph-3* on the susceptibility *per se* of fruit (not determined here) although could also arise from a reduction in the size and sporulation intensity of lesions leading to lower inoculum pressure on fruit in an infected field planting.

#### 4.4.1.3 *Legend and West Virginia 63*

The moderate to large increases in IE seen on these cultivars (and indeed NC2-CELBR and Mountain Magic) between 6 and 11 DPI when inoculated with 13\_A2 and 8\_A1 may be as a result of *Ph-2* delaying the onset of visible symptoms, in line with the reported disease-progress-limiting effect of this gene (Chunwongse *et al.* 2002; Nowicki *et al.* 2013; Turkensteen 1973). Ultimately, high mean IE was seen in both cultivars when inoculated with 13\_A2 and 8\_A1, suggesting that these isolates were able to overcome *Ph-2* in time. Mean SI was also not significantly different from that on Ailsa Craig (susceptible) except in the case of inoculations with the 6\_A1 isolate, for which it was significantly reduced. Overall, the modest reduction in mean LD and the slowing of lesion development probably do not make up for the high ultimate IE and high SI with most isolates. In a field situation these characteristics would be likely to slow the spread of the disease somewhat, but ultimately provide little protection. Although fruit susceptibility was not assessed here, it has been shown to correlate with foliage susceptibility in field situations in trials by Butz (2010) and in the present study (**Chapter 2**). In the field trials described in **Chapter 2**, lines carrying *Ph-2* alone showed only a small reduction in the level of foliage disease compared with susceptible lines. Lines with *Ph-2* showed no reduction in the level of fruit infection compared to susceptible lines in 2013, and only a small reduction in 2012. Lines with *Ph-2* alone were also susceptible in trials in Asia (AVRDC 1995; AVRDC 1998; AVRDC 1999; Black *et al.* 1996a; Black *et al.* 1996b) where *Ph-2* alone no longer provides effective *P. infestans* resistance (Chunwongse *et al.* 2002).

#### 4.4.1.4 *NC2-CELBR and Mountain Magic*

The two cultivars with resistance from both *Ph-2* and *Ph-3* generally showed the strongest performance with regard to all resistance parameters measured here. Although IE by 13\_A2 and 8\_A1 was high, and on that parameter alone these isolates appeared to “overcome” the resistance in these cultivars (**Figure 4.3**), the resulting lesions grew slowly (**Figure 4.4** and **Figure 4.5**) and produced few or no spores (**Figure 4.6**), so the expected spread within a field crop would be low. The observed field performance of NC2-CELBR and Mountain Magic (and other lines carrying both genes) in the field trials described in **Chapter 2** was very much in line with this prediction.

The apparently synergistic effect of combining *Ph-2* and *Ph-3* has been reported by several authors (Brusca 2003; Foolad *et al.* 2008; Nowicki *et al.* 2013) and observed in the field trials described in **Chapter 2**, where analysis of variance identified a significant interaction between *Ph-2* and *Ph-3* genotype. The findings presented here provide a plausible explanation for this synergy, in that *Ph-2* in Legend and West Virginia 63 appeared to delay lesion development and slow growth, whilst *Ph-3* in Plum Regal provided stronger suppression of sporulation. Thus the two genes combined may suppress inoculum production and spread in a field situation more effectively than either alone. However, this is unlikely to be a complete explanation for the apparent synergy, as it does not explain the fact that IE by *P. infestans* isolate *Unique* was significantly depressed on NC2-CELBR, and IE by 8\_A1 was significantly depressed on both NC2-CELBR and Mountain Magic.

#### **4.4.2 Appraisal of methodology**

##### *4.4.2.1 Factors affecting the precision of inoculation experiments*

Since the present experiments were carried out, a comprehensive experimental appraisal of tomato germplasm screening methods (including detached leaflet testing similar to that for this experiment) was published by Nowakowska *et al.* (2014). They report on earlier work by Nelson (2006) investigating the effect of the age of plant material on lesion growth rate, as well as comparing inoculation and assessment methods. The study did not detect any effect of the age of the plant supplying the leaflets, although Nowakowska *et al.* (2014) found that leaflets taken from 4-5 week old plants showed smaller lesions than those taken from 7-8 week old plants. It therefore seems prudent to ensure that plants for *P. infestans* aggressiveness testing are of the same age. The plants used in this study were all sown at the same time and grown alongside each other, so differences in plant age and growing conditions should not have affected the results obtained. Nelson (2006) did however find that the age of leaves from a given plant significantly affected the growth of lesions on tomato leaflets, with older leaves developing significantly larger lesions than younger ones. Furthermore, the position of the leaflet on the compound leaf affected lesion size also, with terminal leaflets developing significantly larger lesions than axial leaflets. Whilst in this study only axial leaflets were employed, following a protocol based on that by Bakonyi and Cooke (2004), the age of leaflets was not controlled beyond ensuring they were fully mature and healthy, and not showing signs of senescence. In future work, the precision of estimates of disease parameters could be improved by ensuring leaflets are of the same age, by, for example, harvesting only the first third to fifth youngest leaves from a relatively young plant (the strategy adopted by Nowakowska *et al.* (2014)).

Different modes of leaflet inoculation (spray vs. droplet inoculation) were also assessed by Nelson (2006), who found that both were equally effective but the droplet method (as employed here) was preferable on account of being quicker, more economical with inoculum, and easier to perform correctly and consistently. The same study also compared three lesion size assessment methods: a pictorial comparison method, measurement using callipers, and measurement using a scanner and computer with image analysis software. They favoured the pictorial comparison method similar to that used here, as they found that it gave acceptable accuracy and was considerably faster than either of the other methods.

In their assessment of the suitability of detached-leaflet inoculations for assessing a cultivar's *P. infestans* resistance, Nowakowska *et al.* (2014) found that for inoculation with a given *P. infestans* isolate, the magnitude of intra-cultivar variability in lesion size varied between *P. infestans* isolates, and they chose an isolate which produced the most consistent results across replicated inoculations onto identical leaf material. In the present study, isolates were chosen based on their (SSR) clonal lineage and high aggressiveness (in terms of mean lesion diameter). The selected isolates all produced high mean values of LD, although when they were chosen, no consideration was given to the consistency (standard deviation) of the disease reaction. In future work, this would be a sensible criterion to consider when choosing isolates for detached leaflet tests or any other germplasm screening. Nowakowska *et al.* (2014) also examined the influence of inoculum concentration ( $5 \times 10^3$ ,  $1 \times 10^4$  and  $5 \times 10^4$  sporangia mL<sup>-1</sup>) on the disease reaction, and found that at the lowest concentration, the reaction of some cultivars was less consistent and therefore deemed concentrations below  $1 \times 10^4$  sporangia mL<sup>-1</sup> unsuitable for resistance evaluation. The concentration used in the present study was  $1 \times 10^4$  sporangia mL<sup>-1</sup>, which is at the lower end of the range of concentrations generally used in other studies, although not unusual (for example Bakonyi and Cooke (2004), Lebreton and Andrivon (1998), Legard *et al.* (1995), and Vleeshouwers *et al.* (1999)). A low concentration was deliberately chosen with the aim of differentiating susceptible from more resistant cultivars on the basis of infection efficiency.

#### 4.4.2.2 Correspondence of laboratory assessments to field performance

A more fundamental question concerns the relevance of detached leaflet testing (with its inherently somewhat artificial nature) to the *P. infestans* resistance of tomato varieties in real horticultural situations. The method has been and is widely used (for example, Bakonyi and Cooke (2004), Kim and Mutschler (2006), Lebreton and Andrivon (1998), Legard *et al.* (1995), and Zhang *et al.* (2014)). However, Foolad *et al.* (2008) state that in their experience, the technique provides a “reasonable” assessment of *P. infestans* resistance, but is “not a fully reliable screening system”. Nowakowska *et al.* (2014) give a more detailed experimental evaluation of the technique, reporting that detached leaflet tests similar to those performed in the present study

showed a significant, but rather weak correlation with field performance of the cultivars tested ( $R^2 = 0.41$ ). They found that detached whole-leaf (as opposed to leaflet) tests offered a far better approximation of field performance ( $R^2 = 0.94$ ), which was as good as or better than the estimate provided by whole plants in growth cabinets (for which  $R^2 = 0.83$ ). Therefore, were this study to be repeated, modifying the protocol to use detached compound leaves rather than leaflets would be a fairly easy step that may increase the reliability of the results. However, Nowakowska *et al.* (2014) did find that more susceptible cultivars exhibited more consistent responses in detached leaflet tests and field trials, and concluded that detached leaflet tests were an effective way to identify susceptible plants (perhaps to eliminate them when selecting germplasm in the early stages of a breeding programme). Although detached leaflet testing has these limitations, it does have a role to play in saving space and time (even over detached whole-leaf testing) when multiple isolates are to be compared, as was necessary in this study. Here, detached leaflet studies did successfully show significant and biologically plausible differences among cultivars and isolates for infection efficiency and sporulation intensity in addition to lesion size.

#### 4.4.2.3 Choice of differential cultivars

Perhaps the greatest improvements to the design of this study would come from changes to the selection of cultivars used as differentials so that a wider range of genotypes and gene combinations could be compared. The most obvious change would be to include cultivars carrying only homozygous *Ph-3* and heterozygous *Ph-2* genes. At the time that the study was conducted, the authors were not aware that any available cultivars had these genotypes. With the benefit of the information on tomato genotypes obtained during the work presented in **Chapter 2**, a number of readily available tomato cultivars could have been used if their genotypes had been known at the time, for example Previa (Graines Voltz) or Ferline (Gautier Semences), both of which were shown to carry heterozygous *Ph-2*, and Rote Murrel (Vreeken's Zaden), which was shown to carry homozygous *Ph-3*. Whilst it would be interesting to examine the effect of combinations of *Ph-1* with other *Ph-* genes, there is no indication in either scientific or grey literature that any cultivars carrying such gene combinations exist. The cultivar Magic Line-Up (New World Seeds and Tubers) from the 2013 field trial was shown to carry homozygous *Ph-2* and heterozygous *Ph-3* (**Chapter 2**). None of the cultivars from the 2013 field trial, which were genotyped using multiple replicates of several different markers, carried heterozygous *Ph-2* with homozygous *Ph-3*, although cultivars from the 2012 trial (which were less reliably genotyped) appeared to have this *Ph-* genotype. Additionally, the partially inbred ( $F_5$ ) line MM6-2 from the 2013 trial had this genotype. Sibling line populations such as the "MM" breeding lines in development (or better still, near-isogenic lines resulting from backcrosses to a susceptible parent) carrying different resistance gene combinations would be the ideal differential lines for



performing an evaluation such as this. It is likely that the differing genetic backgrounds of the cultivars used as differentials in the present study had at least some influence on the *P. infestans* infection parameters independently of the *Ph*- genotype. This appeared to be the case with the two homozygous *Ph-2* cultivars Legend and West Virginia 63, which exhibited significantly different IE with *P. infestans* isolates 8\_A1 and *Unique*, and observable, although not significant, differences in other infection parameters also. This supposition is supported by the fact that potato cultivars with the same (susceptible) resistance gene profile have been shown to nevertheless display some degree of differentiation in their *P. infestans* susceptibility (Colon *et al.* 1995). At present, no tomato near-isogenic differential set exists. Whilst such a differential set would obviously provide an insight into the resistance offered by different *Ph*- genes, it would also have application in phenotypically classifying *P. infestans* races in the same way as the potato *R* gene differential series developed by Malcolmson and Black (1966) and Malcolmson (1969), although with greater relevance for tomato breeders.

## 4.5 Conclusions and Recommendations

This study provides further evidence of the greater strength of *P. infestans* resistance conferred by *Ph-2* and *Ph-3* combined than by either gene alone. By showing that the genes appeared to affect different components of resistance, it also provides some explanation of how the combination of the two genes might reduce inoculum production and thus reduce disease in the field. However, as this “synergistic” reduction in infection efficiency in the laboratory tests was apparent also, reduction in inoculum production and secondary spread is unlikely to be the only mechanism. The study showed that the *Ph-1* gene had little effect on most resistance components, although did appear to slightly reduce infection efficiency by some isolates.

This study was limited in the range of genotypes it included, and there are questions around the degree to which detached leaflet studies such as this can predict cultivars’ field performance. Nevertheless, there is a place for laboratory studies in simultaneously testing cultivars against multiple *P. infestans* isolates, and in dissecting the components of resistance, both of which were achieved in the present study. However, studies such as this could probably be done more reliably and without unduly increasing the cost in time or money by using detached leaves, as opposed to leaflets. A more complete selection of *Ph*-genotype combinations would obviously be desirable, and ideally they would be in a uniform genetic background, although no such tomato differential set exists, and producing one would be valuable, but time-consuming and expensive.

These findings add further weight to the call on breeders to focus on multi-gene *P. infestans* resistance, as much for increased current effectiveness as to improve the durability of resistance. The fact that two of the four *P. infestans* isolates tested readily infected even the most resistant

tomato cultivars further highlights the need to identify further resistance genes from wild populations and germplasm collections.

## 5 QTL mapping in a “Koralik” X “NC2-CELBR” tomato F<sub>2</sub> population

### 5.1 Introduction

#### 5.1.1 QTL identification and mapping

As indicated above, transferring minor-gene resistance to cultivated crop plants is most successful when genetic markers linked to the QTLs of interest are available. Genetic mapping makes use of populations of progeny from crosses between parents with contrasting phenotypes for the trait(s) of interest. The progeny population and parents are genotyped at a number of genetic marker loci (which must be polymorphic between the two parents) spread throughout their genome (or a chromosome or region of interest if the existence and approximate location of the QTL is already known from previous work). At its simplest, genetic mapping consists of dividing the progeny into pools of individuals which have either the Parent 1 or Parent 2 genotype (plus heterozygotes if the population is not inbred) at each marker in turn. The two pools are then tested for significantly different values of the trait of interest. A significant difference indicates that the marker is linked to a QTL controlling the trait of interest (Young 1996).

The more sophisticated Composite Interval Mapping (CIM) procedure employs other (randomly selected) markers as cofactors to account for some of the unexplained variance and increase the sensitivity of the analysis, and also uses the strength of association between the phenotype of interest and multiple genetic markers to estimate the exact position of a QTL within a space between two markers (Rifkin 2012). Progeny may commonly be segregating F<sub>2</sub> offspring or inbred F<sub>6+</sub> lines from a cross between two inbred parents. Inbred lines have the advantage of being reproducible and giving greater power to detect QTL effects owing to all loci being homozygous (Rifkin 2012), although F<sub>2</sub> populations have the advantage of being faster to produce. Whilst heating and artificial lighting would allow tomato plants to be grown year round, producing an F<sub>6</sub> population from inbred parent lines would realistically take a minimum of around three years, and was therefore not possible within the timeframe of this project.

Genetic mapping relies upon an accurate genetic linkage map, giving the order of the markers on each chromosome (Rifkin 2012). A genetic linkage map for a given mapping population is created by using an algorithm such as the Haldane (Haldane 1919) or Kosambi (Kosambi 1944) mapping function to find the marker order requiring the minimum number of genetic recombination events, given the observed combinations of marker alleles in the individual genotypes. The first linkage map of tomato was constructed using just 84 isozyme and RFLP markers (Bernatzky and Tanksley 1986), but was soon followed by a denser map which was constructed with 135 RFLP

markers (Bonierbale *et al.* 1988) and then a dense map using over 1,000 RFLP markers (Tanksley *et al.* 1992). Subsequently, at least 20 further linkage maps of the tomato genome or regions of it have been created, and published on the Sol Genomics Network (Fernandez-Pozo *et al.* 2015). Many of these were constructed using dense SNP markers (for example, the EXPEN 2000, EXPEN 2012, and EXPIM 2012 maps (Sim *et al.* 2012a), all with over 3000 markers). The advent of SNP array technology has allowed large populations of samples to be simultaneously genotyped at many marker loci, greatly reducing the work and cost involved in QTL mapping (Sim *et al.* 2012a).

### 5.1.2 Previously mapped *P. infestans* resistance QTLs

Previous mapping studies have identified *P. infestans* resistance QTLs on all tomato chromosomes (**Table 5.1**), the majority of which come from *S. habrochaites*, in contrast to the widely used resistance major genes *Ph-2* and *Ph-3* and (obsolete) *Ph-1*, all of which were discovered in *S. pimpinellifolium*. Many of the QTLs identified by Brouwer *et al.* (2004) occurred in only one of several experiments in their study, although Brouwer and St Clair (2004) subsequently confirmed the reproducibility of two QTLs on Chromosome 5 and one on Chromosome 11 across multiple tests, and fine mapped these QTLs to narrow windows.

**Table 5.1: Previously mapped *P. infestans* resistance QTLs**

In most studies, markers linked to QTLs were named, but chromosomal locations were not given. In these cases, all maps on the SolGenomics Network database (Fernandez-Pozo *et al.* 2015) were searched for the linked markers, and the highest mapped position of the upper marker and lowest mapped position of the lower marker are given.

Chr.	Position		Origin	Study
	Upper	Lower		
1	0	67	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
1	80	83	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
1	106	129	<i>S. pimpinellifolium</i> PI 270443	Merk and Foolad (2012); Merk <i>et al.</i> (2012)
1	112	145	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
2	10	54	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
2	69	74	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
3	9	49	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
3	57	94	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
4	0	52	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
4	32.9	85.5	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer and St Clair (2004)
4	Top		<i>S. habrochaites</i> LA1777	Li <i>et al.</i> (2011)
4	Bottom		<i>S. habrochaites</i> LA1777	Li <i>et al.</i> (2011)
5	31	80	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
5	63.9	128.5	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer and St Clair (2004)
5	75	119	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer and St Clair (2004)
5	Bottom		<i>S. habrochaites</i> LA2099 Selection MD1	Haggard <i>et al.</i> (2013)
6	0	19	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
6	19	72	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
6	40.4	64	<i>S. pennellii</i> LA716	Smart, Tanksley <i>et al.</i> (2007)
7	55	67	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
7	Top		<i>S. habrochaites</i> LA1777	Li <i>et al.</i> (2011)
8	3	47	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
8	63	84	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
8	Bottom		<i>S. habrochaites</i> LA1777	Li <i>et al.</i> (2011)
9	32	54	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
9	55	81	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
10	2	29	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
10	61	69	<i>Pimpinellifolium</i> "West Virginia 700"	Gallegly and Marvel (1955)
10	67	106	<i>S. pimpinellifolium</i> PI 270443	Merk and Foolad (2012), Merk, Ashrafi <i>et al.</i> (2012)
11	27	55.2	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer and St Clair (2004)
11	27	77	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
11	68.8	107	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
12	7	9	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
12	33	57	<i>S. habrochaites</i> LA2099 Selection MD1	Brouwer <i>et al.</i> (2004)
12	Middle		<i>S. habrochaites</i> LA1777	Li <i>et al.</i> (2011)

### 5.1.3 Mapping population parents

#### 5.1.3.1 Cherry tomato cultivar Koralik

The heirloom cultivar Koralik is a vigorous bush type originating from Poland (Bralewski *et al.* 2006). In earlier trials by Pro Veg Seeds Ltd., it exhibited traits of interest to Pro-Veg, including early cropping, high yield of small fruits, and high sugar level and good flavour (John Burrows, Pro-Veg Seeds Ltd., Sawston, Cambridge, *Pers. Comm.*). Fruits are round cherry-type and those harvested in the present experiment had a mean weight of 2.5 g. (Hallmann and Rembiałkowska 2007; Rembiałkowska and Hallmann 2007) examined the soluble solid and nutrient content (sugars, vitamin C, lycopene, beta-carotene and the flavonoid quercetin) in Koralik and nine other tomato varieties and found that Koralik contained significantly higher levels of all except lycopene (it should be noted that all but one of the other tomato varieties were large fruited, as opposed to cherry tomatoes, and would therefore be expected to contain lower soluble solid concentration). Aside from the fact that it was hoped to produce some useful breeding lines exhibiting some of the desirable traits noted above, the main reason that Koralik was chosen as a parent in the mapping study was that it had previously shown good blight resistance in field trials conducted at Bangor University as part of this project and trials conducted by other workers (see **Chapter 2**). At the time that the study was being designed, Koralik was known not to carry either the *Ph-2* or *Ph-3* *P. infestans* resistance genes, although was subsequently shown in this project to carry *Ph-2* (see **Chapter 2**). No information on Koralik's ancestry could be found, but the unusual vigorous, irregular plant habit and small fruit suggested that it may be derived from a wild *Solanum pimpinellifolium* or *S. lycopersicum* var. *cerasiforme* accession. High levels of *P. infestans* resistance exist in wild populations of both of these species, in many cases conferred by genes other than *Ph-2* or *Ph-3* (Foolad *et al.* 2014; Rodríguez *et al.* 2013). Thus it was hypothesised that cv. Koralik may carry one or more novel resistance genes.

#### 5.1.3.2 Slicing tomato breeding line NC2-CELBR

NC2-CELBR is a tomato breeding line carrying both *Ph-2* and *Ph-3* in a homozygous state, as well as the *Ve* verticillium wilt resistance gene and the *I* and *I2* fusarium wilt race 1 and 2 resistance genes (Gardner and Panthee 2008). Fruits are slightly flattened round slicing type tomatoes typically weighing around 100g (6 cm diameter). Plants are vigorous bush type. The *Ph-2* and *Ph-3* genes in NC2-CELBR are derived from the semi-wild-type cultivar "Richter's Wild Tomato" and *S. pimpinellifolium* accession L3707, respectively (Gardner and Panthee 2008).

#### 5.1.4 Hypotheses and objectives

A conventional approach to identifying *P. infestans* resistance QTLs in an accession such as Koralik would have been to cross it to a completely susceptible line, such as Moneymaker, as opposed to another resistant line such as NC2-CELBR. However, this study had the secondary objective of producing breeding material (for Pro-Veg and Burpee's breeding programmes) carrying fruit quality and *P. infestans* resistance traits from both parents. Since the resistance in Koralik was hypothesised to come from genes other than *Ph-2* or *Ph-3*, it was believed that it would segregate independently of resistance from NC2-CELBR in a recombinant population, and thus it would be possible to map Koralik's resistance in a cross with NC2-CELBR. Therefore, an F<sub>2</sub> mapping population was developed from a cross between Koralik and NC2-CELBR and a genetic mapping study carried out with the aim of locating resistance QTLs from Koralik and fruit quality and yield QTLs from either parent. A supplementary aim was to identify potential breeding lines carrying combinations of resistance genes.

## 5.2 Methods

### 5.2.1 Generation of F<sub>2</sub> intercross population

Three plants of both parents were propagated in a greenhouse as described in **Chapter 4**. Seed was sown in spring 2013. Crosses were made in both directions (i.e. with each parent line used as the male and female parent in different crosses). Flowers were emasculated by removing the anthers (using fine tweezers) just before they began to shed pollen, i.e. just prior to the petals opening. Emasculated female-parent flowers were pollinated by rubbing the anthers of a mature flower of the male parent on the exposed stigma of the emasculated flower. Cross-pollinated flowers were then labelled with paper tags and left to develop fruit. Cross-pollinated flowers were not bagged as this is not normally necessary in the case of tomato crosses as pollinating insects are not attracted to emasculated flowers (Simon Crawford, *pers. comm.*) and additionally the greenhouse in which the plants were grown was largely free from pollinating insects as it was normally kept closed, and ventilated by mesh-guarded fans.

Once fruit had developed by late-summer, F<sub>1</sub> seed was extracted from the fruit and cleaned of seed-gel (which contains chemicals that inhibit germination). Seed was cleaned by mixing the seed and fruit gel mixture with an equal volume of 8 g l<sup>-1</sup> sodium carbonate solution and incubating at room temperature (18-22 °C) for 24-48 hours before washing the seed in a sieve and drying at 40 °C for 24 hours. The seed was then sown promptly, and several dozen F<sub>1</sub> plants produced from crosses in both directions were grown over the autumn and winter of 2013-2014

in a heated and artificially lit greenhouse as described in **Chapter 4**. Throughout this process, seed and plants from individual cross-pollinated fruits was kept as separately labelled batches in order to facilitate the detection of unintended female-parent selfs. Plants from both crosses appeared uniform apart from two plants from the NC2-CELBR female which appeared, on visual inspection, to be NC2-CELBR selfs. The batch that these two plants belonged to was immediately discarded, and subsequently F<sub>2</sub> seed was saved from a single batch of F<sub>1</sub> plants from a Koralik female-parent. Prior to saving seed from F<sub>1</sub> plants, the *Ph-2* and *Ph-3* genotypes of the F<sub>1</sub> plants chosen for advancement, as well as of the two parent lines, were tested using dTG422 and TG328 markers as described in **Chapter 2**, as a further check against female-parent selfs. F<sub>2</sub> seed was harvest and cleaned as described for F<sub>1</sub> seed, and several hundred F<sub>2</sub> seeds were sown and after approximately three weeks, 90 (plus two spare) healthy seedlings were then potted on into 10 cm square pots and grown in a heated and lit greenhouse as described in **Chapter 4**.

## 5.2.2 Genotyping

### 5.2.2.1 DNA extraction

Once they were several weeks old, DNA was extracted from leaflets from the 92 tomato F<sub>2</sub> lines using Qiagen DNEasy Plant Mini Kits (Qiagen, Crawley, Sussex), exactly as described in **Chapter 2**. The concentration of the extracted DNA was adjusted to 50 ng  $\mu\text{L}^{-1}$  and 25  $\mu\text{L}^{-1}$  aliquots were sealed in a 96 well plate and shipped by international courier to TraitGenetics GmbH (TraitGenetics GmbH, Gatersleben, Germany) for SNP genotyping. Genotyping was carried out using the 7,720 locus “SolCAP” SNP array (Sim *et al.* 2012a; Sim *et al.* 2012b). Prior to sending DNA for SNP genotyping, the *Ph-2* genotypes of 8 and *Ph-3* genotypes of 32 arbitrarily chosen F<sub>2</sub> lines (1-8 and 59-90 respectively) were tested using dTG422 and TG328 markers as described in **Chapter 2** to ensure that the *Ph-2* and *Ph-3* genotypes were as expected. The *Ph-3* genotype of all lines was later tested using the TG328 marker.

## 5.2.3 Propagation of plant material

### 5.2.3.1 Production of clones

In mid-April, the 90 F<sub>2</sub> lines were potted into 7-litre pots of Melcourt Silvamix potting compost (Malcourt Industries Limited, Tetbury) and grown in a heated and lit greenhouse as described in **Chapter 4**. Once plants had begun to produce side-shoots, clones of each plant were made by snapping 3 to 4 side-shoots from each plant and standing them in beakers of tap water in a cool, shaded greenhouse to root. When cuttings had produced roots after 7-10 days, they were transferred to 10 cm pots of Melcourt Silvamix potting compost. Once the young plants had an



established root system and had produced healthy foliage, in early May, they were planted out as described below.

### 5.2.3.2 Greenhouse trial

The original seed-grown plants in 7-litre pots were kept in a large, partially climate controlled greenhouse. Automatic ventilation kept the daytime temperature between 20 and 30 °C, and heating maintained a minimum night-time temperature of 7 °C (although the ambient night temperature was normally between 10 and 20 °C during the summer). Plants were watered, fed, and left un-pruned as described in **Chapter 4**. Additionally, in June and August, “Nemasys” *Steinernema feltiae* nematodes (BASF plc, Cheadle Hulme) were applied to the compost according to the manufacturer’s instructions, to treat infestations of fungus gnats (*Bradysia* spp.) which were damaging the plant roots. Additionally, weekly sprays of SB Plant Invigorator (Fargro, Littlehampton, West Sussex) were applied according to the manufacturer’s instructions to treat and prevent powdery mildew (*Oidium neolycopersici* or *Leveillula taurica*). Unfortunately, these health problems severely weakened the plants, and this fact combined with the necessary SB Plant Invigorator applications meant that leaflets for detached leaflet tests were not harvested from these plants. However, yield and soluble solids (Brix) data were still obtained from the greenhouse population (see below).

### 5.2.3.3 Poly tunnel trial design

The purpose of the polytunnel trial was to provide leaf material for detached leaflet experiments, and to assess the yield and soluble solids content of fruit. One plant of each of the 90 F<sub>2</sub> lines (with the exception of line 8, of which no cuttings rooted) was planted through weed-suppressant membrane into the soil in a 5 x 20 m polytunnel with 75 cm between rows and between plants in rows. F<sub>2</sub> plants were arranged in ascending order of their code numbers, followed by one plant of each parent and the F<sub>1</sub> at the end of the fourth row, and three Moneymaker susceptible control plants spread through the trial. An additional Moneymaker plant was placed on each end of the four rows to act as a buffer. Plants were trained up strings and pruned as necessary to enable access to the polytunnel. Irrigation was by drip tape laid beneath the membrane. The plants were not fertilised or sprayed with any pesticides or stimulants. Plants suffered some powdery mildew infection during the main experiment period, but it was light enough that it could be left untreated and clean leaflets could be found for detached leaflet experiments. In view of the pest and disease problems suffered by the greenhouse population, the polytunnel population was used to supply all leaf material for detached leaflet tests.

#### 5.2.3.4 *Field trial design and treatment*

The purpose of the field trial was to test the whole-plant *P. infestans* susceptibility of the F<sub>2</sub> plants in a field setting. The clones remaining after planting the polytunnel trial (in most cases, two) were planted in the outdoor field trial. The layout, plant care, randomisation and assessment protocol were as described for the 2012 field trial in **Chapter 2**. The trial was spray inoculated with a 13\_A2 *P. infestans* isolate on the 25<sup>th</sup> of August, although little or no disease spread was observed until late September.

#### 5.2.4 **Detached leaflet experiment design**

Moist chambers for detached leaflet experiments were set up and inoculated as described for **Chapter 4**. However, the present experiments were conducted in a laboratory rather than a greenhouse, as these experiments were conducted in late summer (July and August 2014) when the greenhouse was too hot to carry out detached leaflet experiments. The laboratory used was a stone-walled former farm outbuilding in which the temperature remained around 18-22 °C. An initial experiment used fluorescent light banks to illuminate the chambers, although this was found to raise the temperature in the chambers several degrees above the ambient room temperature, to 24-28 °C, such that *P. infestans* growth was inhibited. In the experiments described here, illumination was solely by natural daylight from the lab's North-facing windows. Chambers were arranged in four rows of 24 boxes on a central bench (96 total). There was one chamber containing six leaflets for each of the 90 F<sub>2</sub> lines, the two parents and F<sub>1</sub>, and three control chambers containing susceptible tomato Moneymaker leaflets. The F<sub>2</sub> plant chambers were arranged in ascending order of their plant ID numbers (which had been arbitrarily assigned when then the population was generated) with the parents and F<sub>1</sub> chambers together at one end. The three Moneymaker control chambers were placed at the ends and centre of the block.

#### 5.2.5 **Data collection**

##### 5.2.5.1 *Data collection from detached leaflet tests*

Naked-eye inspections of the inoculated leaflets were made from the day after inoculation, paying particular attention to the susceptible control leaflets, which it was assumed would be the first leaflets to begin displaying symptoms. Leaflets bearing established lesions potentially capable of sporulation were examined under a Novex 65x zoom binocular microscope (Euromex Microscopen BV, Arnhem, The Netherlands). Once sporulation was observed on any leaflets (generally beginning with the susceptible controls) more thorough examination, including routine microscopic examination of developing lesions, began. The following measurements were made:

- Infection Efficiency data was collected as described in **Chapter 4**.
- Lesion size was recorded at one or two points several days after inoculation (**Table 5.2**). The length of each lesion (along its longest axis) and its width (at the widest point 90° to the length) were measured to the nearest 1 mm using a ruler.
- Latency, the time between inoculation and first sporulation, was recorded to an accuracy of between one and several days (**Table 5.2**). Where the sporulation status was not obvious to the naked eye, lesions were checked for sporulation under the microscope. In order to qualify as “sporulating”, a lesion had to have at least one sporangiophore bearing one or more discernible sporangia.
- Sporulation intensity was assessed visually and recorded on a scale of 0 to 5, where 0 indicated no visible sporangia anywhere on the lesion, 1 indicated a few isolated sporangiophores, 2-4 indicated increasingly dense sporangiophores, and 5 indicated very dense sporangiophores (touching/interlaced) bearing profuse sporangia. Sporulation Intensity was normally recorded at the same time as Lesion Area (**Table 5.2**).
- 

**Table 5.2: Summary of detached-leaflet experiments.**

A total of 12 traits were assessed in these experiments; the assessments of latency from different days were used to compile a single trait, whereas the lesion area and sporulation intensities recorded on different days were considered distinct traits. In all cases, data pertaining to the three different *P. infestans* isolates were considered as separate traits.

<i>P. infestans</i> isolate	Inoculation date	Assessment dates	Traits assessed
6_A1	4 <sup>th</sup> July 2014	5 d.p.i.	Latency
		6 d.p.i.	Latency
		7 d.p.i.	Latency, Lesion area, Sporulation intensity
		12 d.p.i.	Latency, Lesion area, Sporulation intensity
13_A2	20 <sup>th</sup> July 2014	4 d.p.i.	Latency
		5 d.p.i.	Latency
		6 d.p.i.	Latency, Lesion area
		9 d.p.i.	Latency, Lesion area, Sporulation intensity
8_A1	14 <sup>th</sup> August 2014	3 d.p.i.	Latency
		4 d.p.i.	Latency
		5 d.p.i.	Latency
		6 d.p.i.	Latency
		7 d.p.i.	Latency
		9 d.p.i.	Latency, Lesion area, Sporulation intensity

#### 5.2.5.2 *Data collection from field trial*

Disease severity was assessed as in 2012 and 2013 field trials (**Chapter 2**) Two assessments of foliage disease severity took place, the first on the 9<sup>th</sup> of October and the second on the 28<sup>th</sup> of October 2014. Fruit disease severity was only assessed on the 9<sup>th</sup> of October, as most fruit had dropped off the plants by the 28<sup>th</sup> of October.

#### 5.2.5.3 *Fruit data collection from greenhouse and polytunnel*

Fruit yield and quality were recorded for the greenhouse and polytunnel populations. Harvests were performed whenever ripe fruit had accumulated (at one to two week intervals). Harvests were made from the greenhouse population on the 25<sup>th</sup> of July, 4<sup>th</sup> of August, and 10<sup>th</sup> of August. Harvests were made from the polytunnel population on the 28<sup>th</sup> of July, 10<sup>th</sup> of August and 18<sup>th</sup> of August. The following data were recorded:

- The number of fruit harvested from each plant.
- The total weight of fruit harvested from each plant on a harvest day.
- The soluble solid concentration (°Brix) was measured. Only tomato fruit judged to be ripe was harvested. As with the assessment of sporulation intensity, the judgement of ripeness was inherently somewhat subjective, but again, slight inconsistencies between different harvests was relatively unimportant provided judgements of ripeness were reasonably consistent between lines within a harvest, which was easier to achieve. Three tomato fruits (judged to be the least, intermediate, and most ripe (red) of the fruits harvested) were chosen. Juice was squeezed from the fruits by hand and the soluble solid content of the juice measured using an Atago PAL-1 digital refractometer (Atago Co. Ltd., Tokyo) according to the manufacturer's instructions. In cases where no ripe fruit were harvested, this trait was recorded as missing.

An additional final harvest of all remaining ripe fruit was made on the 30<sup>th</sup> of August, although on this occasion soluble solids concentration was not measured.

Throughout these experiments, assistance was provided by two undergraduate students and one MSc student working in the author's lab group. Since assessing the sporulation intensity, and selecting ripe fruit required a degree of subjective judgement on the part of the investigator, these judgements were made by James Stroud only. Placement students assisted by weighing fruit, performing soluble solids measurements on fruits selected by James Stroud, measuring lesion size, and assessing presence or absence of sporangioophores for calculation of latent period.

## 5.2.6 Data analysis

### 5.2.6.1 Processing of detached leaflet data

The recorded lesion dimensions were used to calculate the area of each lesion, assuming the lesion was an ellipse:

$$\text{Lesion Area} = \pi \times \frac{\text{Length}}{2} \times \frac{\text{Width}}{2}$$

Mean values for latent period, lesion area and sporulation intensity were calculated for each individual genotype. The six (or fewer) raw data from each tomato genotype in each detached leaflet experiment were used to produce a mean latency for each F<sub>2</sub> or control genotype in each experiment. Where no leaflets had become infected, all trait data apart from infection efficiency (which by definition equalled zero) were considered missing for that tomato genotype.

### 5.2.6.2 Processing of field trial data

Foliage AUDPC values were calculated as described in **Chapter 2**, based upon the disease severity and 0% infection at inoculation on the 25<sup>th</sup> of August. Since fruit disease data were recorded on one date only, this trait was mapped as % infection.

### 5.2.6.3 Processing of fruit yield and soluble solids data

Data relating to plants in the greenhouse and polytunnel were kept separate, but processed in the same way. The total yield (in grams) from each plant was calculated by summing all harvest weights over the season, as was the total number of fruit harvested. The mean soluble solids content of the fruits tested was calculated for each day a harvest was made (i.e., data recorded at different times were not pooled). In a few cases, no or only one or two ripe fruit were harvested. In these cases, data was recorded as missing if no harvest was made, or the raw value for a single fruit or mean of two values was used, respectively.

### 5.2.6.4 Marker refinement

Data was received for genotypes at 7,720 marker loci, notated in IUPAC nucleotide codes. The nucleotide codes were converted to parental genotypes (coded as “K”, “N”, or “H”, indicating Koralik homozygous, NC2-CEBR homozygous, or heterozygous respectively). The dataset was then screened, and markers were discarded if they failed to meet the following criteria:

- They had been detected successfully for both parent lines, and were polymorphic between them.

- They exhibited a useful level of polymorphism within the F<sub>2</sub> population (as opposed to extreme bias towards one or the other parental genotype).
- They exhibited few amplification failures with the F<sub>2</sub> population.

This resulted in the selection of 2,022 SNP loci as potentially suitable for use in the mapping study. As this number still exceeded what was necessary or easily processed using the available mapping software and computer hardware, a subset of 533 markers with an average spacing of 2.1 cM (**Table 5.3**) were selected (see below).

#### 5.2.6.5 Linkage map

Marker order was derived from two high resolution SNP marker linkage maps (EXPEN2012 and EXPIM2012) from Sim *et al.* (2012a). These published maps were used to produce a consensus linkage map for this population with all 2,022 suitable markers, with distances in cM from the top each chromosome based on Sim *et al.*'s maps. Where more than one SNP had identical genotypes at all 90 lines (i.e. co-located SNPs) only one was retained in the file.

It was apparent from visual inspection of the SNP genotypes in the Excel spreadsheet in which data was manipulated that short regions of Chromosome 11 appeared out of place when they were positioned in the order in which they appeared on Sim *et al.*'s (2012a) map (**Figure 5.1**). Since simultaneous recombination events at the same location in most or all individuals are extremely unlikely, and the apparently mismapped regions could be parsimoniously were not clearly linked to the majority of Chromosome 11 markers. They were used to construct an additional linkage group referred to as Chromosome 11a. QTLs mapping to this linkage group could be safely assumed to map to tomato Chromosome 11, although their position on the chromosome could not be ascertained. Some loci on Chromosome 4 also appeared to be mismapped in this population, although as the number of mismapped loci was clearly far smaller, Chromosome 4 was left as a single intact linkage group.



**Figure 5.1: An example of a mismatched section of Chromosome 11**

Each column represents an  $F_2$  line, and each row represents a SNP marker. Markers are arranged (from top to bottom) in consensus map order. The order shown here implies that two recombination events occurred within a few cM of each other at the same positions (either side of the central two SNP markers) in most  $F_2$  individuals simultaneously. This would be extremely improbable, and it is safe to assume that these two SNP markers are mismatched and belong elsewhere on Chromosome 11. Therefore, regions such as this were removed from the map of Chromosome 11 and considered separately.

**Table 5.3:** Summary of markers selected for input to QTL Cartographer mapping software.

Chromosome	No. of markers	Average inter-marker distance (cM)	Length of chromosome (cM)
Ch. 1	44	2.6	111.8
Ch. 2	53	2.1	108.9
Ch. 3	38	2.2	80.9
Ch. 4	73	1.4	99.5
Ch. 5	55	1.5	83.1
Ch. 6	18	3.5	59.5
Ch. 7	45	1.7	74.2
Ch. 8	35	2.1	70.8
Ch. 9	59	1.6	91.9
Ch. 10	30	2.3	65.9
Ch. 11	49	1.8	87.4
Ch. 11a	14	1.1	14.8
Ch. 12	20	3.8	73.1
<b>TOTAL</b>	<b>533</b>	<b>2.1</b>	<b>1021.8</b>

#### 5.2.6.6 QTL detection

QTL detection for all recorded traits was by Composite Interval Mapping (CIM) performed in Windows QTL Cartographer 2.5 (Wang *et al.* 2012). CIM was performed using a walk speed of 1 cM. The default model (Standard Model 6) was used to automatically select up to 5 cofactor markers, with a 5 cM window either side of the putative QTL, by Forward and Backward Regression with  $P_{in}$  and  $P_{out}$  each 0.1. Regions were considered as candidate QTLs if their LOD

score exceeded 2.5. Location (chromosome number and position) and effect size (mean additive effect size and  $R^2$ ) of all candidate QTLs were exported from QTL Cartographer 2.5 as an .eqtl file. The candidate QTLs were grouped into nine trait categories depending on which of the following traits they affected: fruit soluble solids, fruit number, fruit weight, *P. infestans* infection efficiency, *P. infestans* latent period, *P. infestans* lesion area, or *P. infestans* sporulation intensity, *P. infestans* foliage AUDPC, or *P. infestans* fruit disease severity. Microsoft Excel was used to sort candidate QTLs by chromosome and location within each trait category. Sorted candidate QTLs were manually assessed. Candidate QTLs (which all had a LOD score of >2.5) were retained if their  $R^2$  was above 1%, and their LOD score was above 3.0, or if they co-localised with another candidate QTL which had a similar phenotypic effect. For this purpose, candidate QTLs were considered to co-localise if they were centred within 10 cM of each other.

By extension, QTLs were discarded if they were deemed likely to be spurious. This was the case if they were “unreplicated” QTLs, i.e., QTLs detected on one date and in one population (greenhouse or polytunnel) only, with a LOD score less than 3.0.

## 5.3 Results

### 5.3.1 Verification of cross

After commencement of this experiment, marker genotyping carried out as part of tomato germplasm trialling (**Chapter 2**) had shown that Koralik did in fact carry homozygous *Ph-2*, although not *Ph-3*. Genotyping using markers TG328 and dTG422 in the present experiment confirmed that the  $F_1$  cross carried homozygous *Ph-2* and heterozygous *Ph-3*, as expected in light of the parental genotypes.  $F_2$  lines 1-8 were homozygous for *Ph-2*, as expected, and lines 59-90 segregated for *Ph-3* in a ratio 17 heterozygous, 6 homozygous susceptible, and 9 homozygous *Ph-3*, which was not significantly different from the expected 16:8:8 ratio ( $\text{Chi}^2 = 0.688$ ,  $\text{DF} = 2$ ,  $P = 0.709$ ).

### 5.3.2 Allele Frequencies

Assuming random assortment of alleles in the  $F_2$  population, the expected Mendelian ratio of genotypes would be 50 % heterozygotes and 25 % each parent. Across all 7,720 loci, the observed allele frequencies were 52.8 % heterozygotes, 24.0 % Homozygous Koralik and 23.2 % Homozygous NC2-CELBR. Whilst small, this deviation is significant ( $\text{Chi}^2 = 616$ ,  $\text{DF} = 2$ ,  $P < 0.001$ ).

### 5.3.3 QTLs affecting *P. infestans* Resistance

Composite interval mapping identified 47 chromosome regions which had an effect on *P. infestans* resistance components which was significant at  $\text{LOD} > 3.0$ , or at  $\text{LOD} > 2.5$  in more than one experiment (**Table 5.4**). In the case of 32 of these, the NC2-CELBR allele conferred *P. infestans*



resistance, whilst in 15 cases the Koralik allele conferred resistance. QTLs are named in **Table 5.4** using a nomenclature of the form *function-chromosome<sub>x</sub>*, where <sub>x</sub> is a letter distinguishing multiple QTLs on one chromosome affecting the same trait. In five instances, resistance QTLs based on data for multiple traits were identified at the same location, coming from the same parent; in these instances, the QTLs were given the same name where *function* was “Res” indicated resistance on more than one measure. QTLs were identified on all chromosomes except 1, 6, and 8, as highlighted below.

#### 5.3.3.1 Resistance QTLs on Chromosome 2

Four QTLs from Koralik and two from NC2-CELBR were identified on Chromosome 2. Most QTLs were spread across the chromosome at differing locations, although *13la-2* and *8lp-2* (conferring resistance from Koralik) co-localised at the bottom end of Chromosome 2.

#### 5.3.3.2 Resistance QTLs on Chromosome 3

A single QTL (*Fruit-3*) conferring a small reduction in fruit disease severity from Koralik, was identified on Chromosome 3.

#### 5.3.3.3 Resistance QTLs on Chromosome 4

Five distinct regions conferring resistance were identified on Chromosome 4; one from Koralik and four from NC2-CELBR. Three of these were detected on single occasions only, however two (*Res-4<sub>a</sub>* located between 33.5 and 43.7 cM, and *Res-4<sub>b</sub>*, located between 63.9 and 76.7 cM, both conferring resistance from NC2-CELBR) were identified in multiple measurements of different traits. *Res-4<sub>b</sub>* had a low heritability of only 2%. However, *Res-4<sub>a</sub>* had heritability estimates ranging from 31-58%.

#### 5.3.3.4 Resistance QTLs on Chromosome 5

Two QTLs were identified on Chromosome 5, one from each parent.

#### 5.3.3.5 Resistance QTLs on Chromosome 7

One resistance QTL from Koralik and seven from NC2-CELBR were identified on Chromosome 7. Of the seven QTLs from NC2-CELBR, two colocalised to the region between 37.9 and 41.9 cM, and four colocalised to the region between 46.7 and 55.9 cM.

#### 5.3.3.6 Resistance QTLs on Chromosome 9

Six distinct regions conferring resistance were identified on Chromosome 9. The last of these regions, designated *Res-9*, extended from 74.6 to 85.1 cM and affected nine distinct traits.

Additionally, QTL *13la-9* mapped just above this region. Two QTLs at the top of Chromosome 9 conferred resistance from Koralik.

#### *5.3.3.7 Resistance QTLs on Chromosome 10*

Chromosome 10 carried one resistance QTL from NC2-CELBR and three from Koralik. None of the QTLs affected more than one resistance trait.

#### *5.3.3.8 Resistance QTLs of Chromosome 11*

Two resistance QTLs, both affecting single resistance traits only, were conferred by Koralik.

#### *5.3.3.9 Resistance QTLs on Chromosome 12*

A single QTL for resistance from NC2-CELBR was identified on Chromosome 12.

**Table 5.4: QTLs affecting *P. infestans* resistance**

All chromosome regions associated with *P. infestans* resistance traits at LOD = 3, or LOD > 2.5 in more than one experiment, are listed below. *Add.* = additive effect of the NC2-CELBR allele, therefore where the sign is negative, the Koralik allele increases the value of the trait at that locus. QTL names consist of a code for the trait, followed by the chromosome on which the QTL is located, and a letter if the chromosome bears more than one distinct QTL.

Trait Name	Chr.	SNP	Res. Donor	Pstn. (cM)	LOD	NC2 Add.	R <sup>2</sup>	QTL	2.5 LOD CI (cM)	
									Min	Max
DS Fruit 9.10.14 (%)	2	solcap_snp_sl_26072	K	10.7	3.17	3.72	0.15	<i>Fruit-2</i>	5.5	10.7
Inf. Eff. 8A1	2	solcap_snp_sl_20340	NC2	21.5	3.36	-0.123	0.19	<i>8ie-2</i>	13.6	38.6
Fol. AUDPC (% Days)	2	solcap_snp_sl_10552	K	38.6	3.01	13.7	0.05	<i>Fol-2</i>	38.1	39.6
Min Lat. Perd. 8A1 (days)	2	solcap_snp_sl_15574	NC2	47.0	3.27	0.512	0.29	<i>8lp-2</i>	44.5	49.5
Lsn. Area 6dpi 13A2 (mm <sup>2</sup> )	2	solcap_snp_sl_20063	K	102.0	3.21	46.2	0.27	<i>13la-2</i>	99.1	104.7
Min Lat. Perd. 8A1 (days)	2	solcap_snp_sl_29351	K	106.9	3.21	-1.61	0.17	<i>8lp-2</i>	83.5	107.9
DS Fruit 9.10.14 (%)	3	CL015971-0495	K	36.2	3.56	4.34	0.16	<i>Fruit-3</i>	35.5	39.1
Inf. Eff. 13A2	4	solcap_snp_sl_21335	NC2	17.1	3.27	-0.141	0.10	<i>8ie-4</i>	14.6	24.4
Lsn. Area 9dpi 8A1 (mm <sup>2</sup> )	4	solcap_snp_sl_16978	NC2	39.2	3.61	-112.9	0.31	<i>Res-4<sub>a</sub></i>	35.5	42.5
Mean Lat. Perd. 8A1 (days)	4	solcap_snp_sl_16978	NC2	39.2	4.10	0.861	0.44	<i>Res-4<sub>a</sub></i>	34.5	43.7
Min Lat. Perd. 8A1 (days)	4	solcap_snp_sl_16978	NC2	39.2	4.55	1.11	0.58	<i>Res-4<sub>a</sub></i>	33.5	43.7
Spor. Int. 7dpi 6A1	4	solcap_snp_sl_51374	NC2	45.1	4.93	-0.381	0.35	<i>6si-4</i>	45.1	51.1
Lsn. Area 9dpi 8A1 (mm <sup>2</sup> )	4	solcap_snp_sl_2179	K	72.1	2.51	3.10	0.19	<i>8la-4</i>	72.1	72.1
Spor. Int. 9dpi 13A2	4	solcap_snp_sl_3096	NC2	70.8	3.26	-0.291	0.02	<i>Res-4<sub>b</sub></i>	63.9	76.7
Lsn. Area 6dpi 13A2 (mm <sup>2</sup> )	4	solcap_snp_sl_11515	NC2	76.7	4.54	-37.8	0.02	<i>Res-4<sub>b</sub></i>	76.2	76.7
Min Lat. Perd. 13A2 (days)	5	solcap_snp_sl_48847	NC2	5.0	2.89	0.502	0.16	<i>Res-5</i>	3.0	6.0
Inf. Eff. 8A1	5	solcap_snp_sl_12210	K	71.3	5.35	0.011	0.05	<i>8ie-5</i>	71.3	71.3
Min Lat. Perd. 8A1 (days)	7	SL20017_699_CL009238-	K	27.1	3.10	-0.482	0.02	<i>8lp-7</i>	26.1	27.1
Lsn. Area 9dpi 8A1 (mm <sup>2</sup> )	7	solcap_snp_sl_53598	NC2	41.8	5.01	-22.7	0.09	<i>Res-7<sub>a</sub></i>	37.9	41.8
Mean Lat. Perd. 8A1 (days)	7	solcap_snp_sl_53598	NC2	41.8	3.02	1.08	0.26	<i>Res-7<sub>a</sub></i>	41.8	41.8
Min Lat. Perd. 13A2 (days)	7	solcap_snp_sl_53552	NC2	46.7	3.17	0.406	0.18	<i>Res-7<sub>b</sub></i>	46.7	49.7
Inf. Eff. 6A1	7	CL016543-	NC2	49.7	4.30	-0.188	0.21	<i>Res-7<sub>b</sub></i>	48.7	55.6
Lat. Perd. 13A2 (days)	7	CL016543-	NC2	49.7	3.54	0.429	0.19	<i>Res-7<sub>b</sub></i>	46.7	55.9
Spor. Int. 9dpi 8A1	7	solcap_snp_sl_6291	NC2	55.6	3.76	-0.633	0.41	<i>Res-7<sub>b</sub></i>	48.7	55.6
Fol. AUDPC (% Days)	7	solcap_snp_sl_37060	NC2	73.3	5.17	-127.3	0.24	<i>Fol-7</i>	67.5	74.0

*Continued overleaf*

Table 5.4 (Continued)

Trait Name	Chr.	SNP	Res. Donor	Pstn. (cM)	LOD	NC2 Add.	R <sup>2</sup>	QTL	2.5 LOD CI (cM)	
									Min	Max
Min Lat. Perd. 6A1 (days)	9	solcap_snp_sl_9553	K	22.6	2.63	-1.38	0.02	<i>6lp-9a</i>	22.7	23.5
Min Lat. Perd. 6A1 (days)	9	solcap_snp_sl_16649	K	28.4	3.54	-1.26	0.02	<i>6lp-9b</i>	26.5	30.4
Lat. Perd. 6A1 (days)	9	solcap_snp_sl_22831	NC2	34.4	2.79	0.205	0.09	<i>6lp-9c</i>	33.4	35.4
Lat. Perd. 8A1 (days)	9	solcap_snp_sl_22831	NC2	36.4	3.81	1.27	0.49	<i>8lp-9</i>	34.4	37.4
Lat. Perd. 6A1 (days)	9	solcap_snp_sl_69978	NC2	70.2	5.25	1.75	0.16	<i>6lp-9d</i>	66.4	70.8
Lsn. Area 9dpi 13A2 (mm <sup>2</sup> )	9	solcap_snp_sl_69669	NC2	73.6	2.95	-59.2	0.19	<i>13la-9</i>	73.6	75.6
Inf. Eff. 13A2	9	solcap_snp_sl_69669	NC2	76.6	4.37	-0.176	0.29	<i>Res-9</i>	74.6	86.0
Lat. Perd. 13A2 (days)	9	solcap_snp_sl_69669	NC2	76.6	4.38	0.522	0.39	<i>Res-9</i>	74.6	83.1
Min Lat. Perd. 13A2 (days)	9	SGN-U312631_snp241	NC2	78.0	2.89	0.507	0.18	<i>Res-9</i>	78.0	88.0
DS Fruit 9.10.14 (%)	9	SGN-U312631_snp241	NC2	78.1	3.02	-5.93	0.15	<i>Res-9</i>	77.6	83.1
Inf. Eff. 13A2	9	SGN-U580771_snp624_solcap_snp_sl_69861	NC2	82.1	4.02	-0.156	0.19	<i>Res-9</i>	74.6	86.0
Lat. Perd. 13A2 (days)	9	solcap_snp_sl_69927	NC2	83.1	6.11	0.570	0.39	<i>Res-9</i>	74.6	83.1
Min Lat. Perd. 13A2 (days)	9	solcap_snp_sl_69927	NC2	83.1	7.61	0.721	0.46	<i>Res-9</i>	78.0	88.0
Spor. Int. 12dpi 6A1	9	solcap_snp_sl_69927	NC2	83.1	3.83	-0.636	0.17	<i>Res-9</i>	82.6	83.1
Spor. Int. 9dpi 8A1	9	solcap_snp_sl_69927	NC2	83.1	3.71	-0.347	0.01	<i>Res-9</i>	82.6	85.1
Min Lat. Perd. 6A1 (days)	10	solcap_snp_sl_16501	K	28.1	2.65	-0.563	0.01	<i>6lp-10</i>	28.1	28.1
Min Lat. Perd. 8A1 (days)	10	CL017204-0355	NC2	30.1	4.39	0.277	0.38	<i>8lp-10</i>	30.1	34.0
Fol. AUDPC (% Days)	10	solcap_snp_sl_3294	K	31.2	3.06	80.8	0.03	<i>Fol-10</i>	31.2	31.2
Inf. Eff. 6A1	10	solcap_snp_sl_14850	K	46.3	6.33	0.168	0.31	<i>6ie-10</i>	46.3	46.3
Inf. Eff. 13A2	11	solcap_snp_sl_62616	K	25.2	4.24	0.154	0.21	<i>13ie-11</i>	24.1	30.2
Lat. Perd. 6A1 (days)	11	solcap_snp_sl_28810	K	49.0	3.86	-2.09	0.04	<i>6lp-11</i>	41.0	49.9
Inf. Eff. 13A2	12	solcap_snp_sl_31275	NC2	50.3	3.13	-0.046	0.11	<i>13ie-12</i>	46.3	53.0

### 5.3.4 QTLs affecting fruit soluble solids content

In most cases, alleles from Koralik conferred higher soluble solids content (Brix), the exception to this rule being a single region on Chromosome 5 (**Table 5.5**). In all cases, the additive effect was slight (<1%).

#### 5.3.4.1 Soluble Solids QTLs on Chromosome 3

One QTL of small effect (0.068 °Brix,  $R^2 = 5\%$ ) from Koralik was identified on Chromosome 3.

#### 5.3.4.2 Soluble Solids QTLs on Chromosome 4

One QTL from Koralik was identified on Chromosome 4. This QTL had the largest effect (0.897 °Brix,  $R^2 = 39\%$ ) identified.

#### 5.3.4.3 Soluble Solids QTLs on Chromosome 5

Data from three Brix measurements (the Greenhouse population on the 25<sup>th</sup> of July, and the Polyunnel population on the 28<sup>th</sup> of July and 4<sup>th</sup> of August) supported the existence of a QTL between 57.2 and 60.4 cM where the NC2-CELBR allele conferred higher Brix (although with low heritability –  $R^2 = 2$  to 4 %). An additional QTL for high Brix from Koralik was located at the bottom of Chromosome 5.

#### 5.3.4.4 Soluble Solids QTLs on Chromosome 7

Two distinct QTLs conferring higher Brix were identified on Chromosome 7, both from Koralik. *Brix-7<sub>b</sub>* was identified from the measurement in the Polyunnel population on the 18<sup>th</sup> of August, whilst *Brix-7<sub>a</sub>* was identified in the Polyunnel population on the 4<sup>th</sup> and 18<sup>th</sup> of August. Both QTLs had a moderate additive effect and heritability.

#### 5.3.4.5 Soluble Solids QTLs on Chromosome 9

Two QTLs conferring high Brix from Koralik were identified on the top half of Chromosome 9 (between 35.4 and 46.9 cM). *Brix-9<sub>b</sub>* was strongly supported by data from the Greenhouse and Polyunnel populations on the 4<sup>th</sup> of August, and the Polyunnel on the 18<sup>th</sup> of August. The additive effect of *Brix-9<sub>b</sub>* was 0.436 to 0.613 °Brix with a moderate heritability of 26 to 40 %. A nearby second QTL was identified in the Polyunnel population on the 4<sup>th</sup> of August.

#### 5.3.4.6 Soluble Solids QTLs on Chromosome 11

A QTL near the bottom of Chromosome 11 was supported by data from the Polyunnel on the 28<sup>th</sup> of July and the Greenhouse on the 4<sup>th</sup> of August. The QTL had a relatively strong effect (0.561-0.749 °Brix) and relatively high heritability (60.3 to 85.5 %).

**Table 5.5: QTLs affecting fruit soluble solids (Brix)**

All chromosome regions associated with fruit soluble solids (Brix) traits at LOD = 3, or LOD > 2.5 in more than one experiment, are listed below. *Add.* = additive effect of the NC2-CELBR allele, therefore where the sign is negative, the Koralik allele increases the value of the trait at that locus. QTL names consist of a code for the trait, followed by the chromosome on which the QTL is located, and a letter if the chromosome bears more than one distinct QTL.

Trait Name	Chr.	SNP	High Brix Donor	Pstn. (cM)	LOD	NC2 Add.	R <sup>2</sup>	QTL	2.5 LOD CI (cM)	
									Min	Max
GH Mean SS 10.8 (°Brix)	3	CL017416-0406	K	56.5	4.09	-0.068	0.05	<i>Brix-3</i>	49.9	58.4
GH Mean SS 10.8 (°Brix)	4	solcap_snp_sl_24150	K	54.0	4.25	-0.897	0.39	<i>Brix-4</i>	42.7	56.9
GH Mean SS 25.7 (°Brix)	5	solcap_snp_sl_22597	NC2	59.1	3.98	0.417	0.02	<i>Brix-5a</i>	57.2	60.4
Tun Mean SS 4.8 (°Brix)	5	solcap_snp_sl_22597	NC2	59.1	3.00	0.294	0.03	<i>Brix-5a</i>	59.1	59.1
Tun Mean SS 28.7 (°Brix)	5	solcap_snp_sl_22600	NC2	59.4	3.75	0.336	0.04	<i>Brix-5a</i>	59.1	60.4
Tun Mean SS 28.7 (°Brix)	5	solcap_snp_sl_12210	K	71.3	3.15	-0.305	0.36	<i>Brix-5b</i>	71.3	71.3
Tun Mean SS 4.8 (°Brix)	7	solcap_snp_sl_15780	K	17.5	3.50	-0.285	0.16	<i>Brix-7a</i>	6.0	25.1
Tun Mean SS 18.8 (°Brix)	7	SL10853_1329	K	23.6	3.15	-0.316	0.13	<i>Brix-7a</i>	16.5	23.6
Tun Mean SS 18.8 (°Brix)	7	solcap_snp_sl_31924	K	61.5	4.92	-0.471	0.11	<i>Brix-7b</i>	50.7	62.9
Tun Mean SS 4.8 (°Brix)	9	solcap_snp_sl_22831	K	35.4	3.14	-0.395	0.24	<i>Brix-9a</i>	35.4	37.4
Tun Mean SS 4.8 (°Brix)	9	SGN-U318140_snp404_solcap_snp_sl_39725	K	42.8	8.29	-0.467	0.40	<i>Brix-9b</i>	40.8	45.7
Tun Mean SS 18.8 (°Brix)	9	solcap_snp_sl_39722	K	43.8	5.44	-0.436	0.28	<i>Brix-9b</i>	42.8	45.7
GH Mean SS 4.8 (°Brix)	9	solcap_snp_sl_69429	K	46.9	3.90	-0.613	0.26	<i>Brix-9b</i>	44.3	46.9
Tun Mean SS 28.7 (°Brix)	11	SL1_00sc6004_392552_solcap_snp_sl_56324	K	60.3	2.64	-0.749	0.35	<i>Brix-11a</i>	60.3	62.3
GH Mean SS 4.8 (°Brix)	11	SL10890_654	K	64.3	2.82	-0.561	0.19	<i>Brix-11b</i>	64.3	85.5

### 5.3.5 QTLs affecting fruit number

Five QTLs which affected the total number of fruits harvested from a plant were identified (**Table 5.6**). In four cases, the Koralik allele increased the number of fruits harvested, and in one case, the NC2-CELBR allele increased the number. The QTL from on Chromosome 3 from NC2-CELBR had the strongest effect, increasing by 15.4 the average number of fruits harvested over season, with a heritability of 39%.

Three QTLs from Koralik were located in an 11.2 cM region of Chromosome 11 (29.5 to 40.7 cM) but did not colocalise.

### 5.3.6 QTLs affecting fruit mean weight

Six QTLs affecting fruit mean weight (size) were identified (**Table 5.6**). The Koralik allele of QTL *Size-3* increased average fruit size, although in all other cases, the NC2-CELBR increased the average fruit size. Two QTLs on Chromosome 11 (located adjacently at the lower end) had the strongest effect, adding 8.93 and 3.14 grams to the mean fruit weight on average, with heritabilities of 16 and 25 % respectively.

### 5.3.7 QTLs affecting fruit yield

Four QTLs affecting yield were identified (**Table 5.6**). The QTL on Chromosome 3, for which NC2-CELBR increased the yield, had the strongest effect, increasing the mean yield 106 g over the season, with a heritability of 23 %. QTLs from Koralik all had smaller effects (46.5 to 71 grams) with heritabilities of only ~ 1 %.

**Table 5.6: QTLs affecting fruit number, size, and yield**

All chromosome regions associated with fruit soluble solids (Brix) traits at LOD = 3, or LOD > 2.5 in more than one experiment, are listed below. *Add.* = additive effect of the NC2-CELBR allele, therefore where the sign is negative, the Koralik allele increases the value of the trait at that locus. QTL names consist of a code for the trait, followed by the chromosome on which the QTL is located, and a letter if the chromosome bears more than one distinct QTL.

Trait Name	Chr.	SNP	High Yield/No Mean Wt.	Pstn. (cM)	LOD	NC2 Add.	R <sup>2</sup>	QTL	2.5 LOD CI (cM)	
									Min	Max
<i>Fruit Number</i>										
GH Tot. Frt. Num.	3	solcap_snp_sl_25390	NC2	35.5	8.29	15.4	0.39	Num-3	29.5	43.7
GH Tot. Frt. Num.	11	solcap_snp_sl_21022	K	27.1	4.13	-12.1	0.06	Num-11a	24.1	28.1
Tunnel Tot. Frt. Num.	11	solcap_snp_sl_21074	K	35.3	2.87	-7.94	0.16	Num-11b	35.3	36.4
Tunnel Tot. Frt. Num.	11	solcap_snp_sl_9539	K	40.7	2.83	-7.76	0.18	Num-11c	40.7	40.7
Tunnel Tot. Frt. Num.	12	solcap_snp_sl_14428	K	56	2.58	-2.96	0.01	Num-12	55.9	56
<i>Fruit Size (mean weight)</i>										
GH Mean frt. Wt. (g)	3	CL017416-0406	K	54.5	4.47	-2.69	0.05	Size-3	47.9	58.4
GH Mean frt. Wt. (g)	4	solcap_snp_sl_24150	NC2	54	3.1	1.87	0.14	Size-4	54	55.5
Tun. Mean frt. Wt. (g)	5	solcap_snp_sl_40	NC2	57.4	3	0.634	0.05	Size-5	57.4	57.4
GH Mean frt. Wt. (g)	7	solcap_snp_sl_37191	NC2	71.5	3.64	2.47	0.15	Size-7	63.5	73.3
Tun. Mean frt. Wt. (g)	11	SL1_00sc6004_392552_solcap_snp_sl_56324	NC2	60.3	3.66	8.93	0.16	Size-11a	59.3	62.3
GH Mean frt. Wt. (g)	11	SL10890_654	NC2	64.3	5.09	3.14	0.25	Size-11b	62.3	78.3
<i>Total Yield</i>										
GH Total Yield (g)	3	solcap_snp_sl_35650	NC2	34	3.52	106	0.23	Yield-3	31.5	36.2
GH Total Yield (g)	4	CL017321-0298_solcap_snp_sl_47277	K	82.7	2.61	-46.8	0.01	Yield-4a	82.7	82.7
GH Total Yield (g)	4	solcap_snp_sl_47298	K	88.6	2.6	-46.5	0.01	Yield-4b	88.6	88.6
GH Total Yield (g)	11	solcap_snp_sl_20993	K	26.5	4.97	-71	0.01	Yield-11	23.2	35



## 5.4 Discussion

### 5.4.1 Identification of *P. infestans* resistance QTLs

Identification of *P. infestans* resistance QTLs in this F<sub>2</sub> population was probably hampered by the fact that all individuals carried homozygous *Ph-2*, and the effects of any other resistance genes would have been masked by this. However, whilst this would be expected to reduce the number of resistance QTLs detected, up to 35 distinct chromosomal regions associated with *P. infestans* resistance were identified nevertheless (**Table 5.4**). In the case of 15 of these, higher resistance was conferred by the Koralik allele, whilst in the case of the remaining 20 regions, the NC2-CELBR allele conferred resistance.

Resistance QTLs supported by data from multiple traits or measurements were located on Chromosome 4 (*Res-4<sub>a</sub>* and *Res-4<sub>b</sub>*), 7 (*Res-7<sub>a</sub>* and *Res-7<sub>b</sub>*) and 9 (*Res-9*). Large numbers of candidate resistance genes (NB-LRR genes) were identified by Andolfo *et al.* (2014) throughout the tomato genome, including in locations on Chromosomes 4, 7, and 9 broadly corresponding to the locations of the QTLs identified in the present study. Brouwer *et al.* (2004) and Brouwer and St Clair (2004) identified *P. infestans* resistance QTLs on all 12 tomato chromosomes, but note that many were identified under one set of experimental conditions (assessment date, trial location etc.) only. Likewise, most of the resistance QTLs identified in the present study were detected once only. Since in many cases such QTLs have high associated LOD scores, indicating that there is a highly significant correlation between the chromosomal region and trait in question, it is likely that the effects of these QTLs are highly dependent on environmental conditions such as, for example, temperature, the age of the plant material or conditions under which it was grown, and the *P. infestans* isolate. Johnson *et al.* (2012) note that numerous studies report large numbers (10 or more) of QTLs, most of which do not appear to be replicated across studies. To be useful in field situations, QTL resistance needs to be effective across a broad range of environmental conditions. Thus, in the present study, the QTLs identified on Chromosomes 4, 7, and 9 are likely to be the most useful in breeding. QTLs *Res-4<sub>a</sub>*, *Res-7<sub>b</sub>*, and *Res-9* also had heritabilities which were frequently in the region of 20-50 %, which would make them more amenable to transfer into elite breeding lines than *Res-4<sub>b</sub>*, which had a heritability of only 2 %.

*Res-4<sub>a</sub>* may co-locate with *P. infestans* resistance QTLs identified in *S. habrochaites* by Brouwer *et al.* (2004) and Li *et al.* (2011), and *Res-7<sub>b</sub>* may likewise have co-located with a QTL identified by Brouwer *et al.* (2004). *Res-9* may co-locate with a QTL at the bottom of Chromosome 9 identified by Brouwer *et al.* (2004), but is almost certainly actually the *Ph-3* gene, which was segregating in this population. *Ph-3* is located at the bottom of Chromosome 9, in an interval between 66.5 Mbp and 67.5 Mbp (Chen *et al.* 2014). This region is bordered by SNPs solcap\_snp\_sl\_25745 to solcap\_snp\_sl\_63619 in which corresponds to the region of approximately 67.4 cM to 70.7 cM on

the present consensus map. This is somewhat higher up the chromosome than the mapped location of *Res-9*. This may indicate that the markers flanking *Ph-3* were slightly misplaced on the consensus map used in the present study. As expected, *Ph-2* (at the bottom end of Chromosome 10) was not detected, as it was present in all lines so no polymorphism existed in the population to allow mapping.

## 5.4.2 Identification of fruit quality QTLs

### 5.4.2.1 Soluble solids

Nine distinct QTLs affecting soluble solids content were identified on Chromosomes 3, 4, 5, 7, 9, and 11 (**Table 5.5**). *Brix-5<sub>a</sub>*, the only QTL for which the NC2-CELBR allele conferred increased brix, was detected in three separate assessments across both the Poly tunnel and greenhouse, and co-located (approximately) with Brix QTLs identified in an *S. pennellii* X *S. lycopersicum* cross by Causse *et al.* (2004), indicating that it is likely to be a real QTL, but had a heritability of only 2-4 %, limiting its likely usefulness in breeding. Likewise, *Brix-7<sub>a</sub>* was detected in the Poly tunnel population on two dates, and *Brix-9<sub>b</sub>* was detected in the Poly tunnel on two dates and in the Greenhouse on one date, and also co-located with QTLs located on Chromosome 9 identified by Causse *et al.* (2004). *Brix-11<sub>a</sub>* and *Brix-11<sub>b</sub>* were also detected in both the Poly tunnel and Greenhouse populations and located next to each other. The confidence intervals did not overlap in the current analysis, although they may be a single QTL with a “split” peak caused by a mismapped chromosome segment on Chromosome 11.

Detection in multiple locations provides some assurance that the effect being detected is not environmental (for example, reduced soil moisture in one location leading to increased soluble solids content in fruits grown there).

### 5.4.2.2 Fruit number

Unsurprisingly, most QTL alleles increasing fruit number came from Koralik, which bears high numbers of small fruits (**Table 5.6**). A QTL on Chromosome 12 (from Koralik) had a small additive effect with low heritability, whilst a group of three closely-spaced QTLs on Chromosome 11 (also from Koralik) conferred moderate increases in fruit number with low to moderate heritability. Surprisingly, the QTL with the strongest effect on fruit number was from NC2-CELBR (*Num-3*), increasing the mean fruit number over the season by 15.4, with a heritability of 39 %.

### 5.4.2.3 Yield

Of four QTLs identified as affecting yield (i.e., weight of fruit harvested), the only one with a strong effect was *Yield-3* from NC2-CELBR. This QTL co-located (at around 35 cM) with the strong QTL

for fruit number (*Num-3*) from NC2-CELBR. The three QTLs from Koralik all had heritabilities of only ~1 %, and are therefore likely to be of little value in breeding.

#### 5.4.2.4 Fruit size

Despite being obviously related to (and indeed, calculated from) fruit number and total yield, fruit size (mean fruit weight) appeared to be controlled by distinct QTLs. Unsurprisingly, alleles increasing the mean fruit weight mostly came from NC2-CELBR, which was the larger fruited parent. The single QTL for increased size from Koralik, *Size-3*, co-located in the middle of Chromosome 3 with a fruit size QTL identified in an *S. pennellii* X *S. lycopersicum* cross (Causse *et al.* 2004). *Size-11<sub>a</sub>* and *Size-11<sub>b</sub>* were mapped as separate QTLs, although were immediately adjacent and may in fact be a single QTL region including a mismapped section of Chromosome 11.

### 5.4.3 Appraisal of methods used

#### 5.4.3.1 Consensus map

A consensus map was used because the large marker set available was too dense to be mapped using the software with which the authors were familiar (Mapmaker) and two maps constructed with the markers used in this study were available (Sim *et al.* 2012a). Visual inspection of the table of raw recombinant (see raw data in **Supplementary materials**) that in most instances, this was an acceptable solution. However, small regions of Chromosome 4 and larger regions of Chromosome 11 appeared discontinuous and were clearly mismapped. The mismapped markers from Chromosome 11 were ordered into a separate linkage group. Ultimately, no QTLs mapped to this region, although if they had, it would not have been possible to allocate them to a specific position on Chromosome 11. This strategy was probably adequate for a very preliminary survey such as this which was intended merely as a “first look” to ascertain if useful QTLs might exist in either Koralik or NC2-CELBR, but ultimately an accurate linkage map based on the present SNP dataset would need to be constructed for more accurate mapping. Software such as the R package *R/qtl* (Broman *et al.* 2003) is capable of constructing maps with larger numbers of markers than Mapmaker, although unfortunately lack of time to learn to use the software meant that this was not possible within the timescale of this project.

#### 5.4.3.2 Collection of phenotype data

The visual assessment of sporulation intensity was less sophisticated than the lesion-washing method employed in **Chapter 4**, but was much faster, and was felt to be reasonably consistent, at least within data generated by a single assessor on a single day. Whilst some of the assessments

of lesion size and latent period were carried out by undergraduate placement students, all assessments of sporulation intensity were carried out by James Stroud. Moderate scoring inconsistencies between days or between experiments are not of great concern, as data collected on different days was treated as separate traits in the QTL analysis. Conversely, the measurement of lesion size by accurate measurement of dimensions instead of the quick visual assessments performed for **Chapter 4** was perhaps overcomplicated and unnecessarily time-consuming. Nelson (2006) reported that visual assessment with reference to a pictorial scorecard was approximately as consistent as accurate lesion measurement. Perhaps the biggest issue with the collection of trait data was the use of detached leaflets and their limited correlation with real field performance (see **Chapter 4**). All data *P. infestans* resistance trait data deviated significantly from normality (data not shown), not least because all data except that for lesion area was ordinal as opposed to scalar. Deviations from normality may affect the accuracy of QTL mapping (Yang *et al.* 2009), meaning that some of the QTLs identified in the present study could be mismapped or spurious. However, the fact that a number of QTLs were supported by data from multiple experiments within the study is encouraging. Furthermore, this issue of non-normality applies only to the *P. infestans* resistance trait data; data pertaining to fruit yield and quality was approximately normally distributed (data not shown). Ultimately, the issue of non-normality of the *P. infestans* resistance trait data could be helped through the use of the R package *R/qtl* (Broman *et al.* 2003) to perform the mapping analysis, as this software includes provision for mapping ordinal traits, unlike QTL Cartographer.

#### 5.4.4 Applications in breeding

Most *P. infestans* resistance QTLs were identified as coming from NC2-CELBR, as opposed to Koralik. Indeed, in the case of all four resistance QTLs supported across multiple experiments (*Res-4<sub>a</sub>*, *Res-4<sub>b</sub>*, *Res-7<sub>a</sub>* and *Res-7<sub>b</sub>*) resistance was conferred by the NC2-CELBR allele, suggesting that this breeding line carries resistance genes in additions to *Ph-2* and *Ph-3*. Koralik appears not to be a particularly useful resistance donor unless any of the stronger QTLs (*13la-2*, *8lp-2*, *6ie-10*, *13ie-11*) proved to be more widely effective across a range of conditions than the present study suggests they are.

Brix QTLs from Koralik on Chromosomes 4, 7, 9 and 11 confer useful increases of 0.285 to 0.897 °Brix. The Brix QTLs on Chromosomes 9 and 4 were mapped to different locations than the resistance gene *Ph-3* and QTLs *Res-4<sub>a</sub>* and *Res-4<sub>b</sub>* (identified here). Koralik supplied few useful QTLs for yield or fruit size or number, aside from three QTLs for small increases in fruit number (with modest heritability) on Chromosome 11.

## 5.5 Conclusions and recommendations

Despite its methodological flaws, the present study identified (with a reasonable degree of confidence) potentially useful QTLs for *P. infestans* resistance on Chromosomes 4 and 7 of NC2-CELBR, in addition to the known *Ph-2* and *Ph-3* genes on Chromosomes 10 and 9, respectively. QTLs providing useful increases in soluble solids content were identified on Chromosomes 4, 7, 9, and 11 of Koralik. Reasonable heritability and effect size means that these QTLs could potentially have applications in breeding programmes. In order for effective use to be made of these QTLs, inbred lines carrying them would need to be developed. F<sub>3</sub> seed was saved from the F<sub>2</sub> mapping population, and could be used to develop recombinant inbred lines for future work and breeding, although this was outside the scope of the present study.

## 6 General discussion

This research project was broad in scope, with two distinct foci: the study of the *P. infestans* population, and also the development of tomato cultivars with resistance to it. However, these aims were broadly complimentary in many regards, and useful answers were obtained to questions in both areas.

### 6.1 Significance of findings

The *P. infestans* survey described in **Chapter 3** uncovered some unexpected and surprising findings, i.e. that the *P. infestans* population in Britain does not appear to be structured by host species, but is highly diverse. This study materially advanced the state of knowledge of the composition of the British *P. infestans* population, as previous surveys based mostly on sampling from commercial potato crops (Cooke *et al.* 2007; Cooke *et al.* 2014; Cooke *et al.* 2003; Day *et al.* 2004) had described a population dominated by the 13\_A2 and 6\_A1 clonal lineages, with low frequencies of other genotypes. The findings of this work were published in the journal *Plant Pathology* in 2015 (Stroud *et al.* 2015). The findings described in **Chapter 3** are in clear contrast to the situation described with regard to commercial potato crops, with a diverse range of *P. infestans* genotypes present on both host crops (tomato and potato) in British gardens and allotments. This finding may indicate that sexual recombination occurs more frequently in gardens and allotments than it does in commercial fields, leading to the more diverse, less obviously clonal population which was observed. The lack of apparent structure in the populations surveyed, either on the basis of host, geography, or other factors, is surprising. It is unfortunate that a thorough phenotypic examination of the isolates collected could not be undertaken as part of this project, and such work in future could provide more conclusive answers to the question of whether host-specialisation takes place in Britain than were obtained through this study. The role of gardens and allotments in generating and spreading *P. infestans* inoculum has been hotly debated by gardeners and potato industry bodies (Ball and Stevenson 2012) although the present findings do seem to lend support to the view that gardens and allotments are sources of *P. infestans* diversity. However, the fact that the commercial crop has been dominated by a handful of successful clonal lineages for several years suggests that spread of novel genotypes is rare. This may be because commercial potato crops are usually protected by either fungicides or *P. infestans* resistance genes, or both, as well as generally good biosecurity, and the *P. infestans* lineages which infect commercial crops are only those with some ability to overcome these crop defences, whilst gardens and allotments provide a haven for less virulent

*P. infestans* genotypes, and those more sensitive to fungicides. Nevertheless, the potential exists for gardens and allotments to serve as a “nursery ground” in which virulent and aggressive isolates may arise. Indeed, in screening of isolates for use in the detached leaflet experiments described in **Chapter 4**, it was a garden-derived “Unique” isolate (presumably a recombinant) which was the most aggressive isolate on most measures, illustrating the potential of garden-derived novel isolates to be potent disease-causing agents. Ultimately, what these findings suggest is a need for measures to reduce the incidence of *P. infestans* in British gardens and allotments. Part of this may come through improved biosecurity procedures by gardeners, although this is unlikely to be the whole answer. Given that gardeners are understandably unwilling or unable to use fungicides, increased availability of tomato and potato cultivars with genetic resistance to *P. infestans* seems to be the most viable way of achieving this aim.

The breeding work described in **Chapter 2** materially advanced this aim, as to date one novel tomato cultivar with high blight resistance conferred by *Ph-2* and *Ph-3* combined has been released from this work, under the name “Crimson Crush”. The variety has been on sale to amateur gardeners from British seed merchants since the beginning of 2015. Whilst at the time of writing, the 2015 tomato cropping season, and blight-outbreak season, had not yet begun, initial feedback from gardeners has been extremely positive, and demand for Crimson Crush has matched or exceeded the currently available seed supply (Simon Crawford, *pers. comm.*). The results of the field trials described in **Chapter 2** (and the detached leaflet experiments described in **Chapter 4**) add to the weight of evidence (Brusca 2003; Foolad *et al.* 2014; Nowicki *et al.* 2013; Wagner 2012) that a combination of *P. infestans* resistance genes confers the strongest resistance. Furthermore, the experiments presented in **Chapter 4** provided some evidence of a residual effectiveness of the *Ph-1* gene, at least in providing a slight reduction in infection efficiency by some isolates. Given the “synergistic” effect of combining the *Ph-2* and *Ph-3* genes, it seems that an attempt to combine *Ph-1* with other resistance genes (perhaps by crossing an OP cultivar carrying *Ph-1*, such as New Yorker, to lines with *Ph-2* and/or *Ph-3*) would be instructive, especially if they were tested against a broad range of *P. infestans* genotypes in the laboratory (as in **Chapter 4**) as well as in outdoor field trials.

Combined *Ph-2* and *Ph-3* have been shown in field trials conducted in this project and elsewhere (Brusca 2003; McGrath *et al.* 2012; McGrath *et al.* 2013; McGrath and LaMarsh 2014) to provide adequate protection to contemporary *P. infestans* isolates in British and North American field conditions. However, the vulnerability of *P. infestans* resistance in tomato based on only one or two major genes has long been recognised (Foolad *et al.* 2014), and efforts are underway at a number of institutions to identify further resistance genes (see **Chapter 1**). The results presented in **Chapter 4**, showing that even the homozygous *Ph-2* / *Ph-3* line NC2-CELBR was successfully

infected by some isolates, add further support to the argument that additional resistance genes should be found. The genetic mapping study discussed in **Chapter 5** attempted to identify novel resistance QTLs from the cultivar Koralik. In this aim, it was not resoundingly successful, as whilst QTLs conferring *P. infestans* resistance (or components thereof) were detected in Koralik, none of these were replicated in multiple experiments using different *P. infestans* isolates or making measurements at different stages. This suggests that the QTLs detected may have been either spurious, or highly specific to certain experimental conditions such as plant growth stage or *P. infestans* isolate, making them less useful for providing resistance to *P. infestans* in real field situations. However, the study did identify, with greater repeatability, resistance QTLs on Chromosomes 4 and 7 of NC2-CELBR, in addition to the *Ph-2* and *Ph-3* genes known to be present in this line. In order to confirm the existence of these QTLs and their potential usefulness in breeding, a repeated mapping study should be carried out.

The design of the mapping study described in **Chapter 5** was unorthodox in that both parents were resistant to *P. infestans*. The reason for this decision was a hope that breeding lines carrying multiple *P. infestans* resistance genes could be produced during the mapping study. Ultimately, this did not occur, in part because of the undesirable plant habit of most of the F<sub>2</sub> progeny from the Koralik X NC2-CELBR cross. Outsourced genotyping was expensive, and in the context of this PhD project, was the main cost associated with this mapping study (although the cost of genotyping has now come down to the point at which, in a normal commercial situation, phenotyping is often the larger cost (Foolad *et al.* 2008). However, the SNP array genotyping conducted here tested 7,720 markers, of which 2,022 were polymorphic and of sufficient quality to be considered for use. In fact, such a large number of markers was far more than were needed for a preliminary study such as this, where the limiting factor on the quality of the output was the small F<sub>2</sub> population size (90 individuals) and the limited amount of phenotype data that was collected. With hindsight, it would have been better to allocate resources to genotyping more individuals (ideally more than 200) and collecting more and better quality phenotype data (for example, by focussing on an expanded field trial instead of the rather labour intensive detached leaflet assessment). For a preliminary study, a marker spacing of around 10 cM would have been adequate, meaning that the genotyping could have been conducted using around 100-120 markers. Finally, the decision to cross two resistant parents was ultimately counter-productive, as the presence of homozygous *Ph-2* in all individuals, and of at least one copy of *Ph-3* in three quarters of the population, is likely to have hampered QTL detection. Accordingly, this strategy could not be recommended for future work. However, despite this, several potentially useful QTLs (for *P. infestans* resistance as described above, and also for soluble solids on Chromosomes 4, 7, 9 and 11) were detected. Further work to confirm, characterise, and more precisely map these QTLs could be undertaken using F<sub>6</sub> RIL populations developed from the saved F<sub>2</sub> seed. The RILs



would need to be re-genotyped, but as noted above, this could probably be done more cheaply at fewer marker loci. Aside from continued work with the NC2-CELBR X Koralik cross, a mapping study to identify the resistance in other lines tested during this project would be useful. Notably, the cultivar Matt's Wild Cherry performed excellently in field trials (**Chapter 2**). The genetic basis of this resistance was unclear – the results of genotyping for *Ph-3* markers were ambiguous. Other workers have also made note of the resistance shown by this cultivar and its unclear genetic basis (McGrath *et al.* 2012; McGrath *et al.* 2013; McGrath and LaMarsh 2014). At present, no groups appear to be investigating this resistance further, and it could therefore be a worthy topic of future investigation.

## 6.2 Conclusions and recommendations

The survey of *P. infestans* genotypes illustrate that gardens and allotments host a very different population of *P. infestans* isolates to commercial potato crops. This points to the need for an increased focus of monitoring efforts on this *P. infestans* population. The survey did not give a conclusive answer to the main question it sought to address, i.e. whether host specialisation occurs in Britain, and further phenotypic studies would help to determine this. The study did however highlight the need to reduce the occurrence of *P. infestans* on amateur's tomato and potato crops, in order to reduce the frequency of matings and the level of inoculum production in these settings. An effective way of achieving this aim would be through the introduction of more, and better, *P. infestans* resistant tomato and potato cultivars. The development of novel *P. infestans* resistant tomato cultivars would be considerably aided by the availability of more genes and QTLs for *P. infestans* resistance. The mapping study carried out here suggested the presence of previously undiscovered resistance QTLs on Chromosomes 4 and 7 of the tomato line NC2-CELBR, as well as a range of other potential QTLs on this line and the cultivar Koralik. Further work to characterise QTLs such as these, and those in other lines, is urgently needed, as the current resistance based on only two genes, in most cases, seems rather precarious. Reincorporation of the *Ph-1* gene into contemporary breeding programmes may be worthwhile. Finally biotechnology offers the theoretical potential for transferring resistance QTLs and genes from wild tomato backgrounds to cultivated ones more easily than conventional breeding alone. Whilst genetically engineered crops would be unlikely to be enthusiastically received by amateur gardeners at present, this situation may change in time. Whether by genetic engineering or marker-assisted conventional breeding, introgression of multiple *P. infestans* resistance genes into tomato remains a priority, and efforts to discover and map them should continue.

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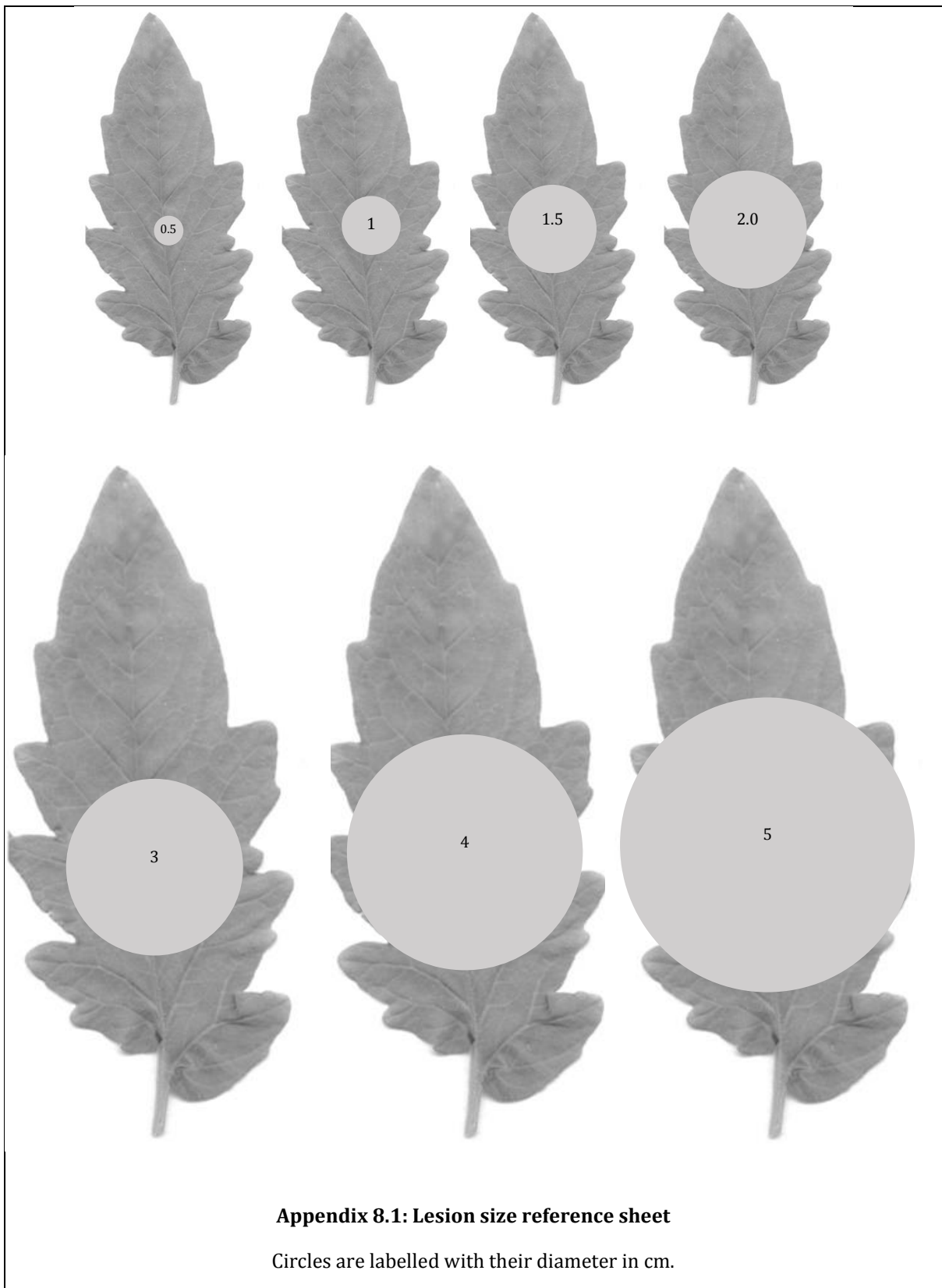
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## 8 Appendices



### Appendix 8.2: Isolate collection aggressiveness pilot

Results of a small pilot study assessing the aggressiveness of 25 *P. infestans* isolates collected in 2013 on detached tomato cv. Ailsa Craig leaflets. Lesion diameter was measured at 6 days post inoculation. Isolate ID does not relate to any wider nomenclature system. Isolates are arranged by increasing mean lesion diameter. Isolates highlighted in bold were used in the pathotyping study. P or T at the beginning of the isolate ID indicates the host of origin (Potato or Tomato).

Isolate ID	Mean Lesion Diameter (cm)	N	Std. Deviation (cm)	Minimum Lesion Diameter (cm)	Maximum Lesion Diameter (cm)
T22	1.25	4	0.29	1.00	1.50
P32	1.50	5	1.12	0.00	3.00
T38	1.50	5	0.00	1.50	1.50
T49	1.80	5	0.27	1.50	2.00
P11	1.88	4	0.63	1.00	2.50
T12	1.88	4	0.85	1.00	3.00
<b>T44</b>	<b>1.88</b>	<b>4</b>	<b>0.48</b>	<b>1.50</b>	<b>2.50</b>
T71	1.88	4	0.25	1.50	2.00
P01	2.00	3	0.00	2.00	2.00
<b>P23</b>	<b>2.00</b>	<b>5</b>	<b>1.17</b>	<b>0.00</b>	<b>3.00</b>
P16	2.13	4	0.63	1.50	3.00
P23	2.21	12	0.86	0.00	3.00
T73.5	2.38	4	0.48	2.00	3.00
All Isolates mixed	2.40	5	1.02	1.50	3.50
T07	2.42	6	1.43	0.00	3.50
T53	2.50	5	1.00	1.50	4.00
T61	2.50	5	1.00	1.50	4.00
T27	2.63	4	0.75	2.00	3.50
P06	2.67	3	0.58	2.00	3.00
P08	2.67	6	1.17	2.00	5.00
23_A1 <sup>a</sup>	2.73	11	1.17	0.00	4.00
<b>T40</b>	<b>2.75</b>	<b>6</b>	<b>0.61</b>	<b>1.50</b>	<b>3.00</b>
T48	2.83	6	0.82	2.00	4.00
P13	2.92	6	0.20	2.50	3.00
P02	3.17	3	0.76	2.50	4.00
<b>T20</b>	<b>3.17</b>	<b>6</b>	<b>0.98</b>	<b>2.00</b>	<b>4.00</b>

<sup>a</sup> Isolate 23\_A1 was supplied as a culture on agar by Dr David Cooke (James Hutton Institute, Invergowrie, Dundee, UK)

## Appendix 8.3:

### Assessment of Pro-Veg Tomato material for commercial production potential, Bangor, 2012

#### 1) Materials and Methods

- a) Support Systems
- b) Germplasm and Site
- c) Fruit Harvests
- d) Blight Assessment
- e) Analysis

#### 2) Results and Discussion

- a) Missing Data
- b) Blight Assessment
- c) Fruit Yields
- d) Labour Requirements

#### 3) General Comments



A trial carried out by James Stroud, with help and advice from Dr David Shaw, Simon Crawford, Barrie Smith and John Burrows, at Henfaes Research Centre, Bangor University. Additional practical help was provided by Will Johnson, Ruby Bye, Mitch Bradley-Williams, Adi Moor, and Marie Madigan



## **Assessment of Pro-Veg Tomato material for commercial production potential, Bangor, 2012**

A growing trial was carried out in summer 2012 to assess the potential of Pro-Veg tomato cultivars for commercial cropping on a small scale, under different production systems, and also to assess the relative blight tolerance of the cultivars. The trial was carried out by James Stroud with help from David Shaw and a number of others at Henfaes Research Centre in North Wales.

### **1) Materials and Methods**

#### *a) Support Systems*

Three growing systems were trialled: outdoor wire supports, indoor wire supports, and outdoor tomato cages (*Fig. 1a-c*). Wire supports consisted of tensioned 80 cm wide pig fencing held 15 cm above the soil surface by wooden bearers. The square lattice ranged from 15x7.5 cm mesh at one side to 15x15 cm mesh on the other. The mesh was underlain with weed-suppressant membrane, covering the entire area in the case of the outdoor trials (*Fig. 1a*) or a 1.05 m wide strip in the case of the indoor trial (*Fig. 1b*) where weed growth was expected to be minimal. Tomato cages were trialled outdoors only, again placed on weed suppressant membrane (*Fig. 1c*). The cages consisted of large square mesh (approx. 20x20 cm).

#### *b) Germplasm and Site*

Trial material consisted of 23 tomato genotypes (Altino, originally to be 18<sup>th</sup> of 24 genotypes, was missing from the consignment of plants provided by Pro-Veg). Most were lines developed by Pro-Veg, but also included material from other breeders (see *Table 1*). The plants treated here as “Koralik” were discovered to be another unknown cultivar which had been accidentally substituted for Koralik at some point in the propagation process. The results are presented as Koralik here, but it should be borne in mind that they are unlikely to be true Koralik plants.

In the case of the cages, a single plant was grown in each cage (1 replicated per genotype). In the case of the wire support systems, 3 plants were placed in a block, approximately 60 cm apart, with approximately 90 cm between blocks. For purposes of harvesting and blight assessment, each block of three was treated as one replicate.

Outdoor systems were on a stony loam, which had previously been pasture and had been ploughed recently. Soil nutrient status was deemed adequate, so no amendments were added. Soil moisture was at field capacity, and in places soil was waterlogged at times, owing to very heavy rainfall. Seed was sown in early May, and plants were transplanted in on the 24<sup>th</sup> to 26<sup>th</sup> of June. Plants were watered heavily when they were first planted, but were not subsequently irrigated.



**Fig. 1a** (left): Outdoor wire supports, which hold tomato foliage 15 cm from the ground. **Fig. 1b** (centre): Indoor wire supports. **Fig. 1c** (right): Outdoor wire cages. Photographs taken late July 2012 (approx. 4 weeks after planting out in late June).

Indoor trials were carried out in a 5.5 m by 20 m polytunnel, which was left open at both ends at all times apart from during periods of very high wind or severe cold. Soil was high in OM and major nutrients owing to very heavy dressings of sheep manure for previous potato crops. The trial was periodically irrigated using drip tape laid in the soil surface under the membrane, although watering was kept to a minimum to increase fruit quality and to help reduce very high vegetative vigour exhibited by many of the cultivars. Paths in the greenhouse were periodically hoed and hand weeded, although owing to the dryness of the exposed soil, weed growth was minimal.

**Table 1:** Tomato genotypes received from Pro-Veg. Note that no. 18 (Altino) was not received.

No.	Cultivar	Female	Male	Source	Description
1	Omer-2	432-7	JA	Pro-Veg	Vigs. cherry fruit. G. taste
2	Omer-4	9031	JA	Pro-Veg	V Vigs mini Plum BR
3	Omer-5	410-7	JA	Pro-Veg	V Vigs mini Plum BR
4	Omer-56	410-7	9042	Pro-Veg	Vigs baby plum long trusses
5	Omer-98-	412-2	9025	Pro-Veg	Vigs, cherry, G. taste BR
6	Losetto			Pro-Veg	LB outdoor staking type control
7	Lizzano			Pro-Veg	LB outdoor staking type control
8	Sweet Zen			Sakata	Baby plum type, trailing habit?
9	Jolly Elf			Siegers	Semi-determinate outdoor type
10	“Koralik”			PVSL	LB outdoor staking type control
11	Omer-49	9042	9031	Pro-Veg	Med Comp Vigs mini Plum BR
12	Omer-54	405-8	9042	Pro-Veg	Comp early cherry cf Terenzo
13	Omer-65	412-2	BP	Pro-Veg	G mid size plum 435 upgrade
14	Omer-67	432-7	405-8	Pro-Veg	Comp early cherry cf Terenzo
15	Omer-68	410-7	405-8	Pro-Veg	Med vigs cherry BR
16	Tumbler			Ball	Trailing control
17	Red Alert			PVSL	Early Bush, outdoor type
18	Altino			Pro-Veg	Trailing control
19	Siderno			Pro-Veg	Bushy vigorous cocktail control
20	Terenzo			Pro-Veg	Trailing control
21	Tumbling Tom Red			Vegetalis	Trailing control
22	Omer-96	9034	9025	Pro-Veg	Dwarf pot type, Bitonto upgrade
23	Omer-99	9037	9025	Pro-Veg	Dwarf pot type, Bitonto upgrade
24	Bitonto			Pro-Veg	Compact control

### c) Fruit Harvests

Fruit harvests were made whenever a quantity of ripe fruit was present. Only usable fruit were harvested (blighted or otherwise damaged fruit was discarded).

To assess the blight tolerance of the plants, the outdoor trials were inoculated with material taken from a nearby potato trial which had been inoculated with the Blue-13 *P. infestans* strain. The polytunnel trial did not form part of the blight tolerance experiment. To perform the inoculation, a spore suspension was prepared by washing spores from infected potato material using tap water. The spore suspension was then applied to the trial plants directly using a watering can and fine rose, on the 22<sup>nd</sup> of September.

#### d) Blight Assessment

Following the first observation of blight lesions on the 29<sup>th</sup> of September, the trial was assessed visually for blight lesions on foliage and fruit, approximately weekly. Foliage and fruit were assessed separately. Where reasonably well developed fruit were present on the plant (marble sized or larger) the numeric percentage with blight lesions was estimated. The percentage of leaf area infected with or destroyed by blight was estimated.

The assessment date on which ripe fruit was first observed, and the number of days to first ripe fruit was calculated, based on a planting date of the 30<sup>th</sup> of June (plants were planted over several days at the end of June).

#### e) Analysis

Area under disease progress curve (AUDPC) was calculated as follows for each of the replicate plants:

$$\text{AUDPC} = \sum 0.5 (s_i + s_{i+1}) (d_{i+1} - d_i) \quad (\text{APS, 2013})$$

Where  $s_i$  = score on  $i^{\text{th}}$  assessment date

$s_{i+1}$  = score on next assessment date following  $i^{\text{th}}$

$d_i$  = days from inoculation to  $i^{\text{th}}$  assessment date

$d_{i+1}$  = days from inoculation to next assessment date following  $i^{\text{th}}$

$D_0$  was taken to be the 22<sup>nd</sup> of September, when plants were inoculated with spore suspension. Since no infection was observed in the trial prior to inoculation, all plants were assigned a score of 0% foliar infection, and 0% fruit infection if fruit were present.

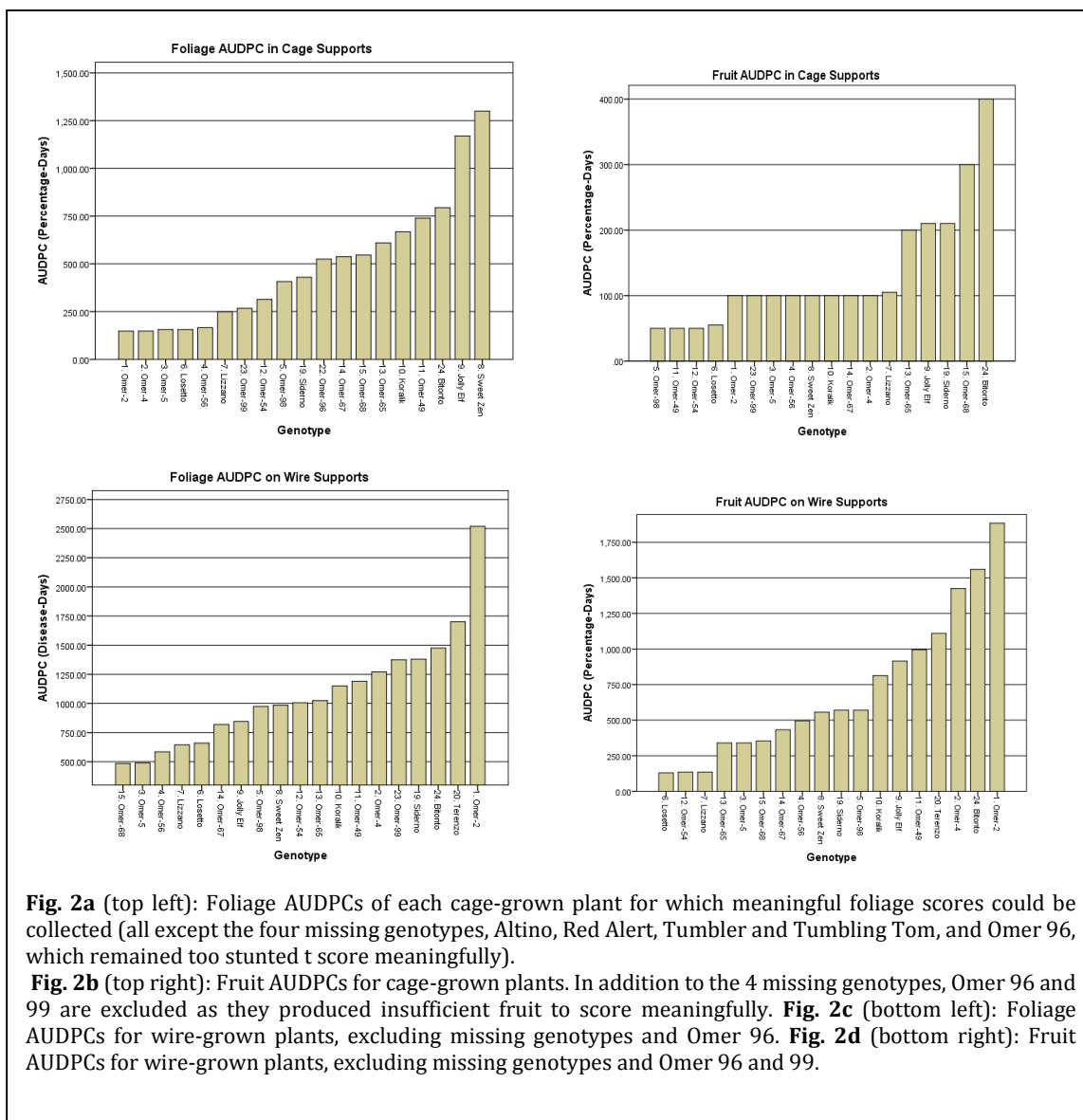
## 2) Results

#### a) Missing Data

Part-way into the trial, *Alternaria* early blight was discovered growing on Tumbler, Red Alert, and Tumbling Tom in the outdoor plantings, so the decision was taken to remove all of these plants from the trial in mid-September to prevent *Alternaria* destroying other plants (the indoor plants were unaffected so were left in place). Also, all Omer 96 and Omer 99 plants remained severely stunted for the duration of the trial (because of the cold, wet weather during much of June and July), and incomplete data was collected for them. Similarly, the cage-grown Terenzo plant was severely stunted, although as this was not the case with the wire-grown plants it may have been due to waterlogging or other localized environmental factors affecting the cage-grown plant.

b) Blight Assessment

Fig. 2 shows the AUDPC for each genotype in each growing system (no error bars are shown as the results are from one replicate only).



It is noteworthy that the evidence here suggests that a genotype's blight tolerance under one system is not a good predictor of its blight tolerance under another; for example, Omer 2 was the most resistant when cage grown, but least resistant when grown on wires. The lack of replication means it is impossible to attach any statistical confidence to any particular result as being "true". However, given that each of the wire-grown results does in fact represent three plants, and also

given that the wire-grown plants were generally larger and better established than the cage grown plants (perhaps due to the slight extra shelter and stabilization afforded by the wire system when plants were establishing) and therefore easier to assess, it seems sensible to attach more weight to these results where there is a contradiction.

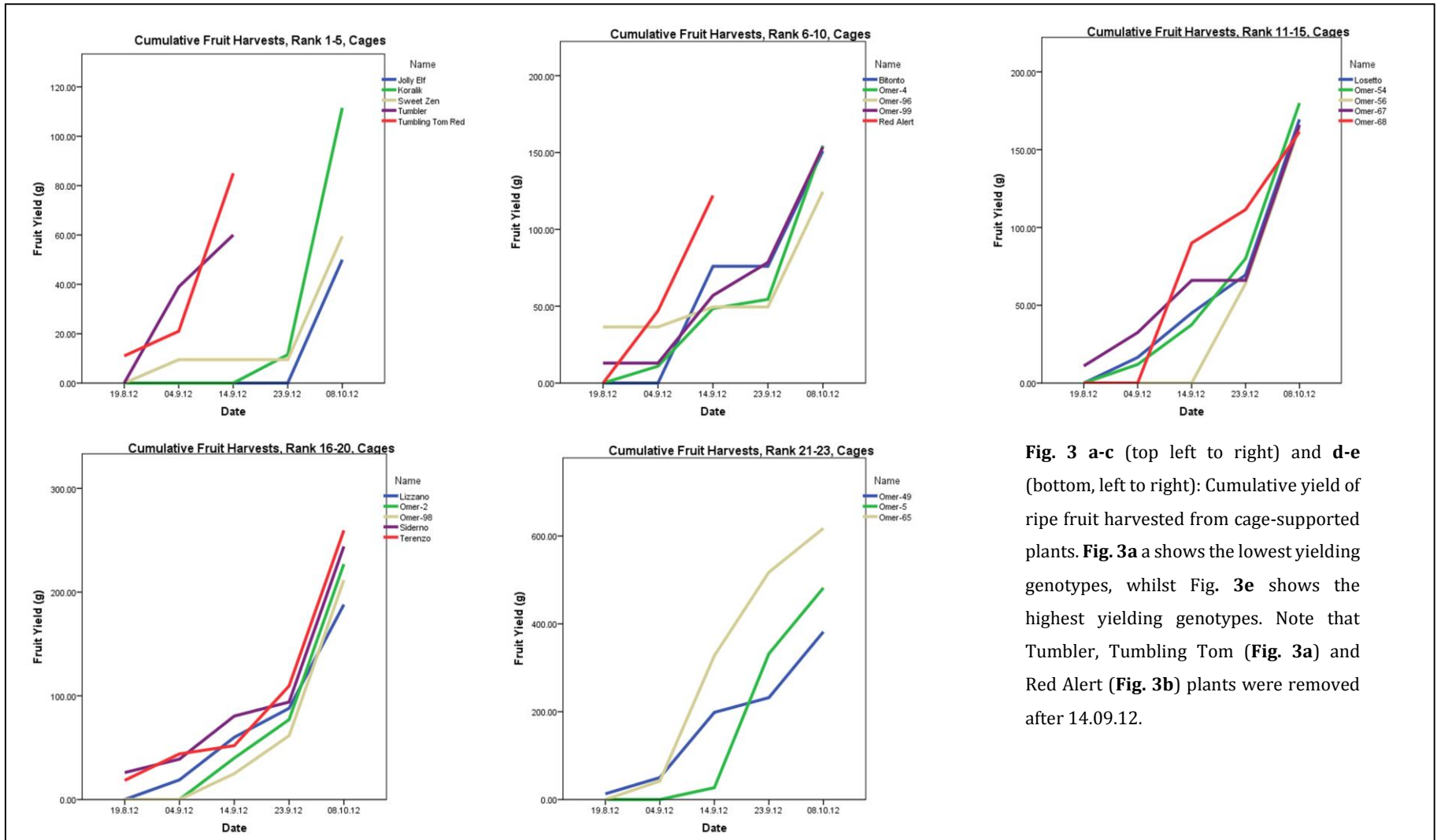
A paired-samples T-test was carried out to establish whether there was a significant difference in blight susceptibility of plants grown under the two systems. Plants grown in cages were found to be significantly less susceptible to blight than those grown on wire supports (*Table 2*). This effect may be due, to some extent, to plants in cages generally being more stunted and therefore actually less susceptible to blight, as foliage was less dense.

**Table 2:** Results of a paired samples t-test comparing foliage and fruit AUDPC for plants grown in cages with those grown on wire supports. AUDPC from 1 cage-grown plant of each genotype was paired with one group of three wire-grown plants of the same genotype.

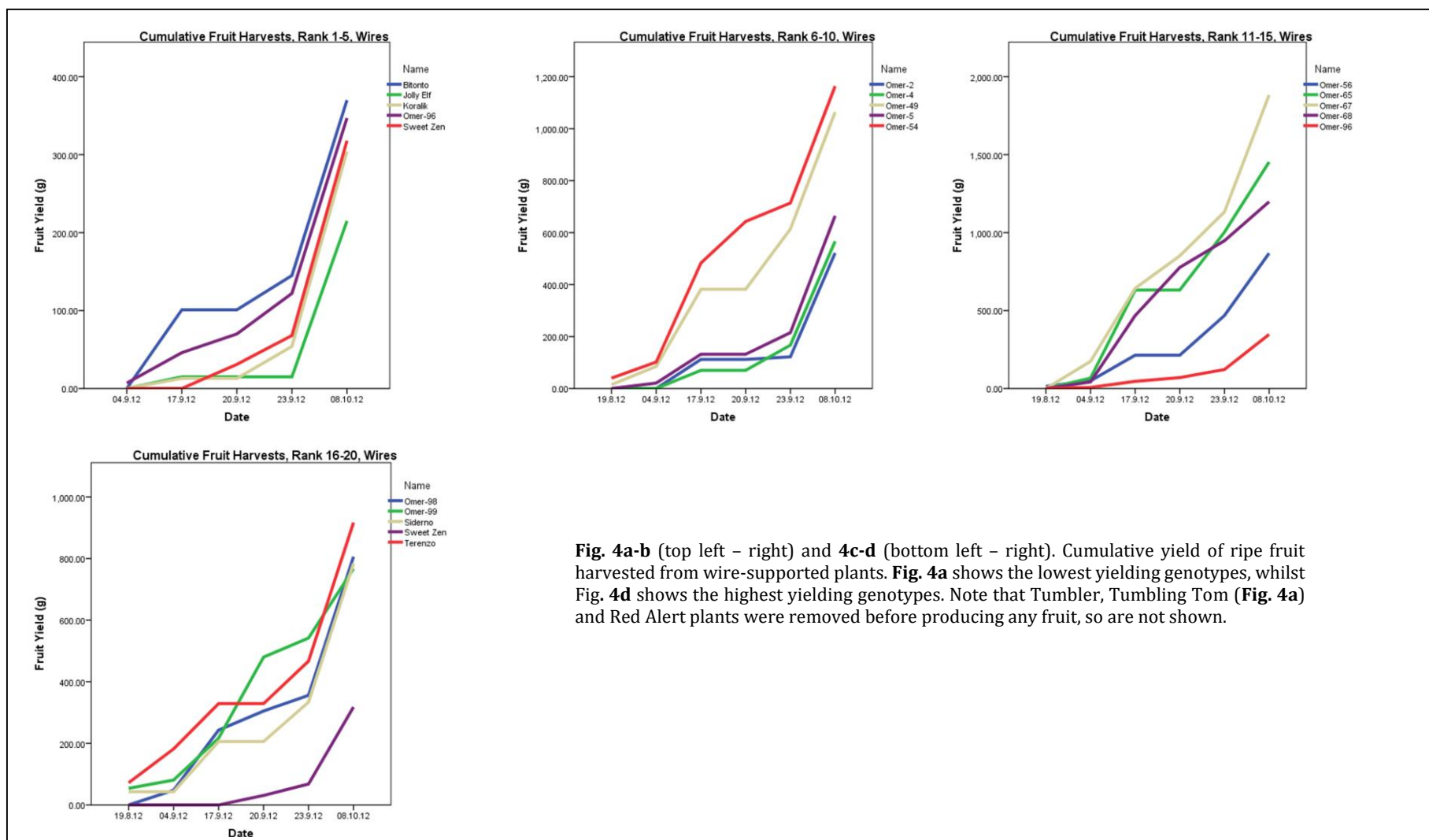
	<b>Wire Mean</b>	<b>Wire Standard Deviation</b>	<b>Cage mean</b>	<b>Cage Standard Deviation</b>	<b>T Statistic</b>	<b>P</b>
<b>Foliage</b>	1029	534	467	345	3.71	0.001
<b>Fruit</b>	638	533	122	98.5	4.48	<0.001

### *c) Fruit Yield*

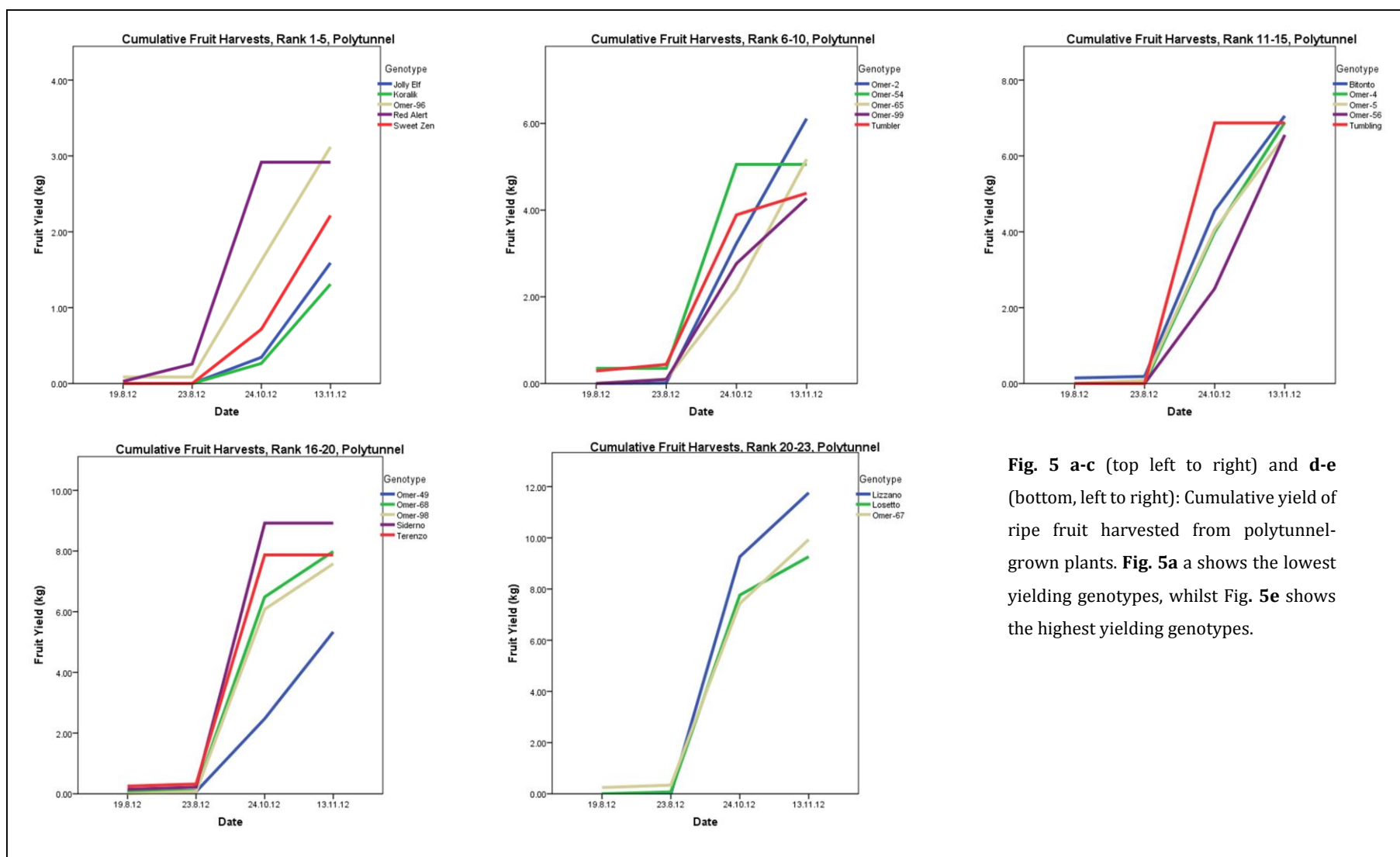
The graphs in *Figs. 3, 4* and *5* show how fruit production varies over time, whilst *Table 3* shows total yields over the growing season.



**Fig. 3 a-c** (top left to right) and **d-e** (bottom, left to right): Cumulative yield of ripe fruit harvested from cage-supported plants. **Fig. 3a** shows the lowest yielding genotypes, whilst **Fig. 3e** shows the highest yielding genotypes. Note that Tumbler, Tumbling Tom (**Fig. 3a**) and Red Alert (**Fig. 3b**) plants were removed after 14.09.12.







**Fig. 5 a-c** (top left to right) and **d-e** (bottom, left to right): Cumulative yield of ripe fruit harvested from polytunnel-grown plants. **Fig. 5a** shows the lowest yielding genotypes, whilst **Fig. 5e** shows the highest yielding genotypes.

Table 3: Total yields per plant over the season. Note that yields given for Tumbler, Red Alert, and Tumbling Tom, are for only part of the season in the case of the outdoor cages.

<b>Genotype</b>	<b>Total Yield per plant from outdoor wires (kg)</b>	<b>Total Yield per plant from outdoor cages (kg)</b>	<b>Total yield per plant from indoor wires (kg)</b>
Omer-2	0.133	0.227	3.00
Omer-4	0.133	0.155	3.26
Omer-5	0.150	0.482	3.00
Omer-56	0.133	0.164	3.54
Omer-98	0.150	0.212	2.53
Losetto	0.267	0.170	3.09
Lizzano	0.233	0.188	3.92
Sweet Zen	0.083	0.060	0.74
Jolly Elf	0.067	0.050	3.00
Koralik	0.083	0.112	3.00
Omer-49	0.150	0.382	3.00
Omer-54	0.150	0.180	1.69
Omer-65	0.150	0.618	1.73
Omer-67	0.250	0.166	3.31
Omer-68	0.083	0.162	2.66
Tumbler	-	(0.060)	1.46
Red Alert	-	(0.122)	0.97
Siderno	0.150	0.244	2.97
Terenzo	0.150	0.260	2.62
Tumbling Tom Red	-	(0.085)	2.29
Omer-96	0.075	0.125	1.04
Omer-99	0.075	0.154	1.42
Bitonto	0.075	0.151	2.35

Fruit yield was generally very considerably higher in the polytunnel than the field. Polytunnel grown plants were generally several times larger and more vigorous than their counterparts in the field, produced many more flowers, and set more fruit. Owing to the fact that they did not

become infected with blight in mid-September, they also cropped for longer. Another very important factor was that the tunnel-grown crops were much less exposed to predation by birds and slugs, which caused considerable losses in the field.

*d) Labour requirements*

The time taken to set up each system, and subsequently manage and harvest from it, is presented in *Tables 4a, b* and *c*. In the case of the outdoor trials, genotypes Red Alert, Tumbler, and Tumbling Tom were removed from the trial before a great deal of time had been spent maintaining or harvesting from them, and these plants are therefore discounted when calculating “per plant” timings for maintenance and harvesting. An intermediate value was chosen to calculate person-hours for the combination of all operations.

**Table 4a:** Labour requirements of outdoor wire supported growing system.

Activity	No. of Plants	Period	Labour (person-hours)	Labour/plant (minutes plant <sup>-1</sup> )	Labour/kg harvested fruit (min kg <sup>-1</sup> )
Site setup and planting	69	Early Summer	27.5	23	195
Maintenance (pruning, training, weeding)	60	Throughout growing season	Minimal (<1)	1	7.1
Harvesting	60	Late summer	5.25	5.3	37
<b>Combined</b>	<b>66</b>	<b>All</b>	<b>33.75</b>	<b>31</b>	<b>239</b>

**Table 4b:** Labour requirements of outdoor cage growing system.

Activity	No. of Plants	Period	Labour (person-hours)	Labour/plant (minutes plant <sup>-1</sup> )	Labour/kg harvested fruit (min kg <sup>-1</sup> )
Site setup and planting	23	Early Summer	3	7.8	73
Maintenance (pruning, training, weeding)	20	Throughout growing season	1	2.9	24
Harvesting	20	Late summer	2.5	7.1	61
<b>Total</b>	<b>22</b>	<b>All</b>	<b>6.5</b>	<b>18</b>	<b>159</b>

**Table 4c:** Labour requirements of indoor wire supported growing system.

Activity	No. of Plants	Period	Labour (person-hours)	Labour/plant (minutes plant <sup>-1</sup> )	Labour/kg harvested fruit (min kg <sup>-1</sup> )
Site setup and planting	69	Early Summer	17.5	15	8.8
Maintenance (pruning, training, weeding)	69	Throughout growing season	1.75	1.5	0.88
Harvesting	69	Late summer	34.75	30	17
<b>Total</b>	69	All	54	47	27

As *Tables 4a-c* show clearly, a substantial part of the time take was in setting up the growing system. Most of this setting up time was spent laying membrane and installing supporting structures, with planting taking only a relatively short time. Therefore, if the system could be left in place and re-used from one year to the next, then the economics of the operation would be improved considerably.

Time spent harvesting per kg of fruit is considerably less in the case of the wire support systems, and especially in the polytunnel. This is likely to be because time spent moving between plants, weighing, and labeling, made up a considerable portion of the total harvesting time. This would remain fairly constant whether a few fruits were harvested from one plant (as was the case in the trial of plant cages), or several kilograms from three (in the case of the wire supports). Furthermore, it was not felt that reaching into the cages greatly increased harvesting time in practice. Additionally, the total time taken per kg of fruit harvested was considerably lower in the case of the polytunnel trial owing to the much higher overall yields in the polytunnel (*Table 3*).

### General Comments

A considerable time was spent establishing the trial, and it may be possible, in a commercial setting, to reduce this considerably, either by re-using the supporting structures from one year to the next, or by using simpler supporting structures. The setup used in this trial was probably over-engineered; it is likely that a less tightly strained, but more closely supported, wire mesh would have been as good as the highly tensioned mesh used here, and considerably easier to build.

The greenhouse-grown plants were extremely vigorous. Generally, no attempt was made to prune the growth, but as harvesting was arguably made more difficult by the excessive mass of foliage, it may well be advisable to keep plants well pruned and limit foliage density.

The yield of usable fruit was reduced to practically nothing from the outdoor plants once they were infected with blight, and given the poor quality of much of the fruit from a field in this state, it is unlikely that it would be worth attempting to harvest from it

However, the yields from the polytunnel grown plants were encouraging. It is likely that harvesting would be quicker if plants were better pruned, and if fruit was not being weighed in small batches as in this trial.

Fruit quality was generally very good in the polytunnel, although less so in the field. Considering the experience of this trial, it would appear that for relatively low-capital commercial production, open polytunnels are likely to be the most successful option, but of course in warmer, drier year, and a commercial field rather than an inoculated trial, unprotected systems may have some potential.

## 9 Supplementary materials

The following Supplementary materials are provided on CD:

- 1) 2011 Field Trial Data (Chapter 2)
- 2) 2012 Field Trial Data (Chapter 2)
- 3) 2013 Field Trial Data (Chapter 2)
- 4) 2014 Field Trial Data (Chapter 2)
- 5) Germplasm identified by James Stroud for the 2012 field trial (Chapter 2)
- 6) Germplasm identified by James Stroud for the 2013 field trial (Chapter 2)
- 7) *Phytophthora infestans* SSR Genotype Table (Chapter 3)
- 8) Detached Leaflet Study data (Chapter 4)
- 9) Mapping Population Field Trial Raw Data (Chapter 5)
- 10) Mapping Population Processed Trait Data (Chapter 5)
- 11) Mapping Population SNP Genotype Table (Chapter 5)