Investigating the virulence of isolates produced by sexual recombination between different *Pyrenophora teres* isolates

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Abstract

Net blotch caused by *Pyrenophora teres* is a major barley (*Hordeum vulgare*) leaf disease in Australia resulting in potential losses of up to 40% to the barley grains industry. It is estimated that this disease costs Australian agriculture $60 million a year. *Pyrenophora teres* occurs as two forms, namely those having net-like symptoms referred to as *Pyrenophora teres* f. *teres* (*Ptt*) and others having spot-like symptoms referred to as *Pyrenophora teres* f. *maculata* (*Ptm*). Progeny have been successfully produced from crossing these two forms in the laboratory and hybrids have also been collected from barley fields. To date the potential evolution of new virulences from crosses between different isolates of the same form and between crosses of isolates of the two different forms has not been investigated. The aim of this study is to a) evaluate a new method (DLA – spray method) for phenotyping net blotch, b) identify the virulences in artificially produced *Ptt* x *Ptt* and *Ptt* x *Ptm* crosses and c) to fine-map the QTL region containing virulence genes in one of these crosses. To achieve this, different virulence assays were trialled and compared to determine which method is the most suitable and reliable. These trials indicated that the DLA – spray method is a reliable and accurate novel method that can replace both the seedling assay and DLA – droplet method for phenotyping net blotch of barley. Virulences were determined in three existing *Ptt* x *Ptm* crosses and one *Ptt* x *Ptt* cross by screening ascospores across a differential set of eight barley varieties. Results indicated that the progeny of these populations express virulences different to their parents'. A genetic map had been developed for *Ptt* x *Ptt* population NB29/NB85 and phenotypic data used to map the virulence genes in this population. For this study a SSR marker was added to this QTL region. Improved knowledge concerning the occurrence of recombination and the potential for new virulences to be produced can be used to better manage disease incursions and to implement control through deployment of resistances.

Declaration

I certify that the work reported in this thesis is entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for assessment in any other course of study at this or any other institution.

Signature of Candidate

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Abbreviations

Abbreviation	Full term
Avr	Avirulence genes
bps	Base pairs
BSA	Bulked Segregant Analysis
DArT	Diversity Arrays Technology
DLA	Detached leaf assay
dsDNA	Double-stranded DNA
EST	Expressed Sequence Tag
HRM	High-resolution melt
MAS	Marker-Assisted Selection
NFNB	Net form of net blotch
OMA	Oatmeal Agar
Path	pathogenic genes
PCR	Polymerase Chain Reactions
PDA	Potato Dextrose Agar
P. teres	*Pyrenophora teres*
Ptm	*Pyrenophora teres* f. *maculata*
Ptt	*Pyrenophora teres* f. *teres*
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
SFNB	Spot form of net blotch
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
ssDNA	Single-stranded DNA
T_M	Melting temperature
V8A	V8 Agar

1 Chapter 1: Literature review

Pyrenophora teres Drechslera (anamorph: *Drechslera teres* [Sacc.] Shoemaker), is the fungal pathogen that causes net blotch disease in barley *(Hordeum vulgare)* (McDonald, 1963). Net blotch is a major barley foliar disease in Australia resulting in potential yield losses of up to 40% to the Australian barley grains industry (Murray & Brennan, 2009).

1.1 Hosts

Pyrenophora spp. is a graminicolous pathogen that affects some economically important genera such as: *Hordeum* genus (barley), *Avena sativa* (oats) and *Triticum aestivum* (wheat), and also other species that are not as important economically, *Bromus* and *Poaceae spp* (Shipton *et al.*, 1973). However, the *Hordeum* genus (barley) is the main host of this pathogen and thus will be the main focus of this study.

As a seed-born disease that can survive on seeds and infested stubble (Bretag, 2009; Shipton *et al.*, 1973; van den Berg & Rossangel, 1991), net blotch can occur wherever barley is cultivated (McLean *et al.*, 2009; Steffenson & Webster, 1992; van den Berg & Rossangel, 1991). However, severe infections occur in temperate regions with high rainfall and humidity (Bretag, 2009; Steffenson & Webster, 1992; Weiland *et al.*, 1999).

1.1.1 Barley production

Barley (*Hordeum vulgare*) is one of the most economically important crops around the world and is grown in Africa, Russia, Canada, USA, Australia and Europe (Fig. 1.1) (Food and Agriculture Organisation, 2005).

Being the second most important cereal crop in Australia after wheat, it is estimated that the average area used for growing barley in Australia between 1998 and 2008 is 3.79 million hectares with an average production of 6.66 million tonnes (Murray & Brennan, 2009).

With the rising demand of high quality grains around the world, the area and production of barley in Australia is massively increasing (Fig. 1.2).

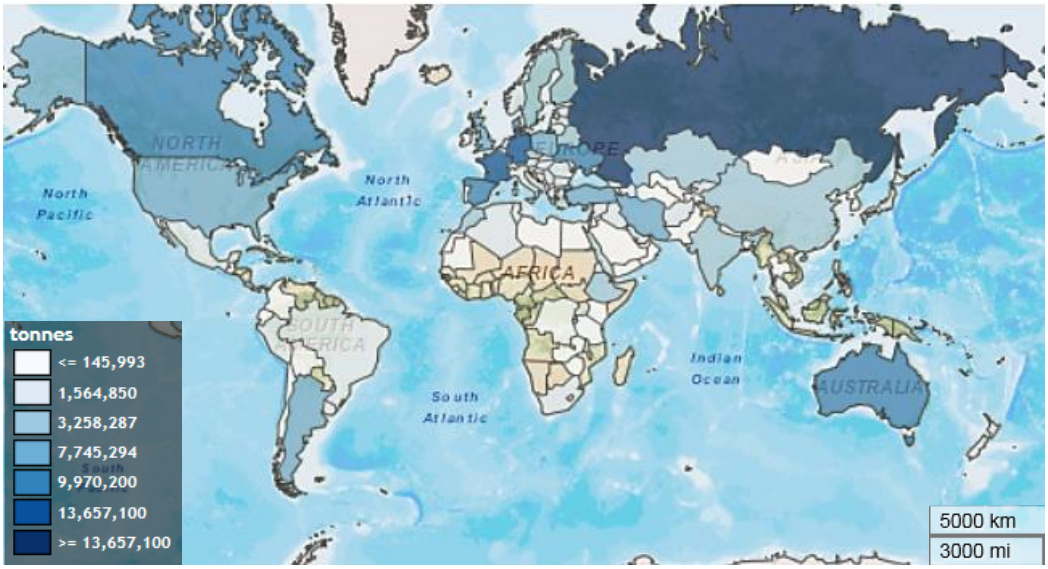


Fig. 1.1 Average barley production (in tonnes) by country _ 2010 – 2013. Sourced from http://faostat3.fao.org/browse/Q/QC/E

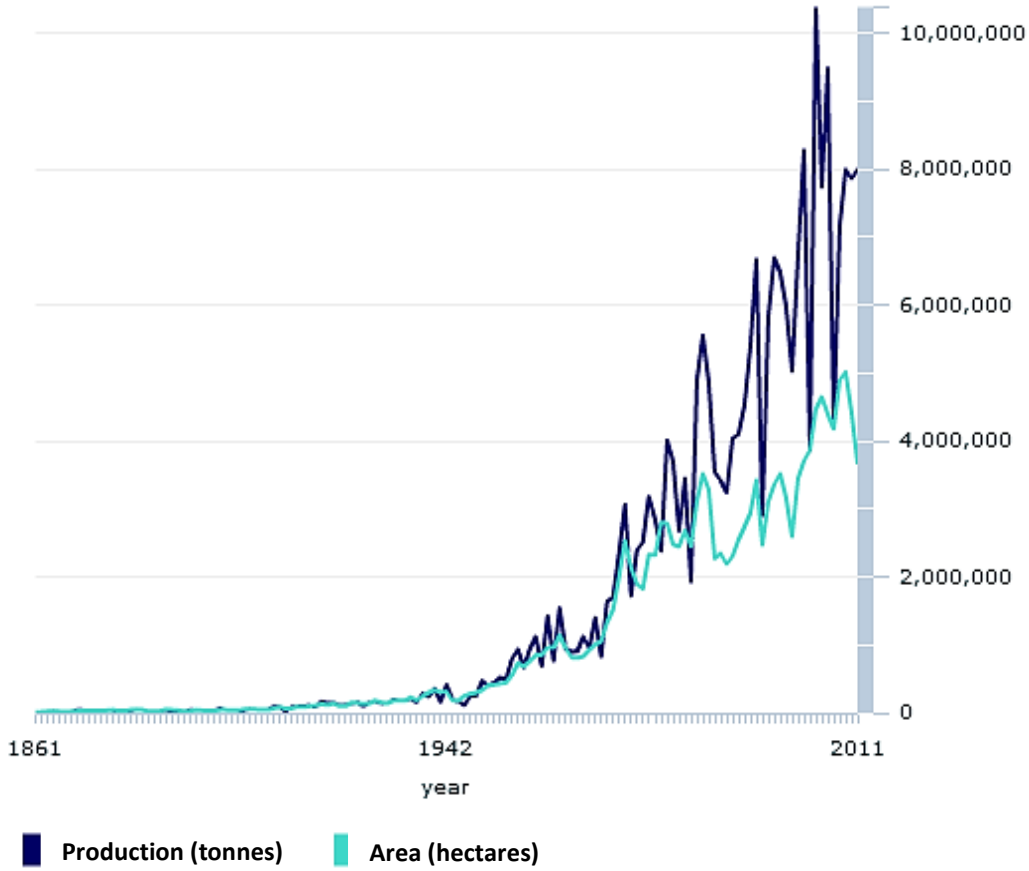


Fig. 1.2 Barley area and production in Australia, 1861-2011 Sourced from: Australian Bureau of Statistics 2015

Barley has multiple uses all of high importance, its grains are used for the livestock feedlot, cereal for human consumption and malted for beer while the straws are mostly used for bedding for animals (Leonard & Martin, 1963).

1.1.2 Economic losses

It is estimated that on average this disease costs Australian agriculture $60 million a year and potentially over $300 million in a high disease year (Murray & Brennan, 2009).

Economic loss is not exclusively due only to yield loss, but also due to the degradation of seed quality. The main reason for the yield loss is the reduction of grain size (Smedegård-Petersen, 1974), a decrease in the ear number, and a reduction in the grain sites per ear (Jordan *et al.*, 1985). Furthermore, the carbohydrate content of grain was found to be reduced (Shipton, 1966).

1.2 Pathogen

While studying net blotch of barley, McDonald (1963) observed that *P. teres* may occur as two forms and concluded that the new form was a mutant. This new form was later found to be another form of *P. teres* that produces different symptoms when interacting with its barley host (Smedegård-Petersen, 1971). The two forms were hence differentiated by Smedegård-Petersen (1971), naming isolates producing net-like symptoms as *P. teres* f. *teres* (*Ptt*) and isolates producing spot-like symptoms as *P. teres* f. *maculata* (*Ptm*). This conclusion was further verified using the Symptom Verification Technique (Crous *et al.*, 1995; Smedegård-Petersen, 1971) and AT- DNA (Louw *et al.*, 1994). For the Symptom Verification Technique, symptoms were verified by inoculating two cultivars: Stirling and B87/14 susceptible to net- and spot-type, respectively. These cultivars were examined within two weeks and the DNA was extracted from the isolates grown in culture to confirm that the spot-type isolate only expressed spot form symptoms and vice versa for the net-type (Crous *et al.*, 1995). Louw *et al.* (1994) used A+T- rich DNA polymorphisms to determine the similarity between the conidial isolates of both spot-type and net-type and ascospore isolates of both spot-type. Bands

between 4 and 21 kb were used to determine the percentage of similarity. The difference between isolates was determined by the presence or absence of individual bands. Louw *et al.* (1994) concluded that the banding patterns of these two pathogens are similar, which verifies Smedegård's (1971) suggestion that the two pathogens are different forms of the same species. The genetic similarity between *Ptm* and *Ptt* according to genetic diversity studies using molecular markers is typically 90 percent (Crous *et al.*, 1995; Lehmensiek *et al.*, 2010). However, multiple phylogenetic studies have demonstrated that the two forms of *P. teres* are genetically isolated and therefore belong to genetically diverse groups (Campbell *et al.*, 2002; Lehmensiek *et al.*, 2010; Rau *et al.*, 2007; Rau *et al.*, 2003; Serenius *et al.*, 2007).

1.2.1 Occurrence

The detection of net blotch of barley around the world was reported in detail by Shipton (1973), but recent publications have been released to further discuss the distribution of net blotch in its two forms. Since the differentiation between net form of net blotch (NFNB) and spot form of net blotch (SFNB) happened by Smedegård (1971), it is assumed that all the detected net blotch incidents before that date were specific to NFNB. Shipton (1973) stated that net blotch was probably first detected in the USA in 1923 (Drechsler, 1923) and the first major outbreak was recorded towards the end of the 1960's. By the 1940's, net blotch was considered the most important seed-borne disease of barley in the United States and Canada where it increased to severe levels in Manitoba, Alberta and the Prairies during the 1950's and 1960's (Buchannon & Wallace, 1962). Other early detections were also recorded in Denmark in 1927 and in Peru in 1929. Despite the rarity of records that document incidence of net blotch in Europe, an epidemic incidence was recorded in Britain in 1930 (Shipton, 1966) and also in Germany in 1934. In Australia, NFNB has existed much longer than SFNB (Khan, 1987). It was first detected in New South Wales in 1948 and then in Western Australia in 1961. SFNB was observed for the first time in Denmark in the 1960s (Smedegård-Petersen, 1971) and then in Finland in 1971 (McLean *et al.*, 2009). In the nineties, SFNB had become epidemic in France

(Arabi *et al.*, 1992). It was first identified in Australian crops at Nabawa in Western Australia in 1977 and has since spread to all barley growing states of Australia including Tasmania (Gupta *et al.*, 2011b; McLean *et al.*, 2010; McLean *et al.*, 2014; Platz *et al.*, 2007).

1.2.2 Pathogen life cycle

No clear differences have been detected in the life cycles of both forms of net blotch (Liu et al., 2011). *P. teres* has the ability to infect many parts of the host (*Hordeum vulgare*) including leaves, leaf sheaths, stems and kernels and can survive on stubble and seed (Shipton et al., 1973). *P. teres* is a polycyclic pathogen that produces more than one infection cycle per crop cycle. Those several asexual cycles are responsible for the spread of the disease (Murray, 2008; O'Brien, 2005). *P. teres* reproduces both sexually and asexually (McDonald, 1963). During sexual reproduction, 1-2 mm, dark globule pseudothecial (Fig. 1.3) fruiting bodies develop on stubble. Within each pseudothecium, club-shaped and bitunicate asci develop. Each ascus contains eight haploid ascospores (Fig. 1.4) which are light-brown with 3-4 transverse and 1-2 longitudinal septa (Mathre, 1997).

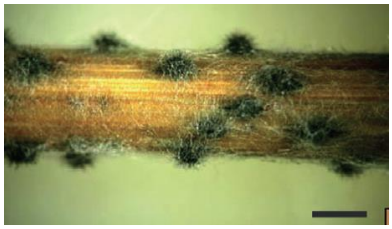


Fig. 1.3 *P. teres* pseudothecia growing on barley straw (scale bar 2.5 mm) (Liu *et al.*, 2011)

Fig. 1.4 *P. teres* ascospores (scale bar 20µm) (Liu *et al.*, 2011)

Upon maturation, ascospores are ejected and dispersed by wind or rain-splash to infect seedlings and serve as primary inoculum (Fig. 1.5) (Jordan, 1981).

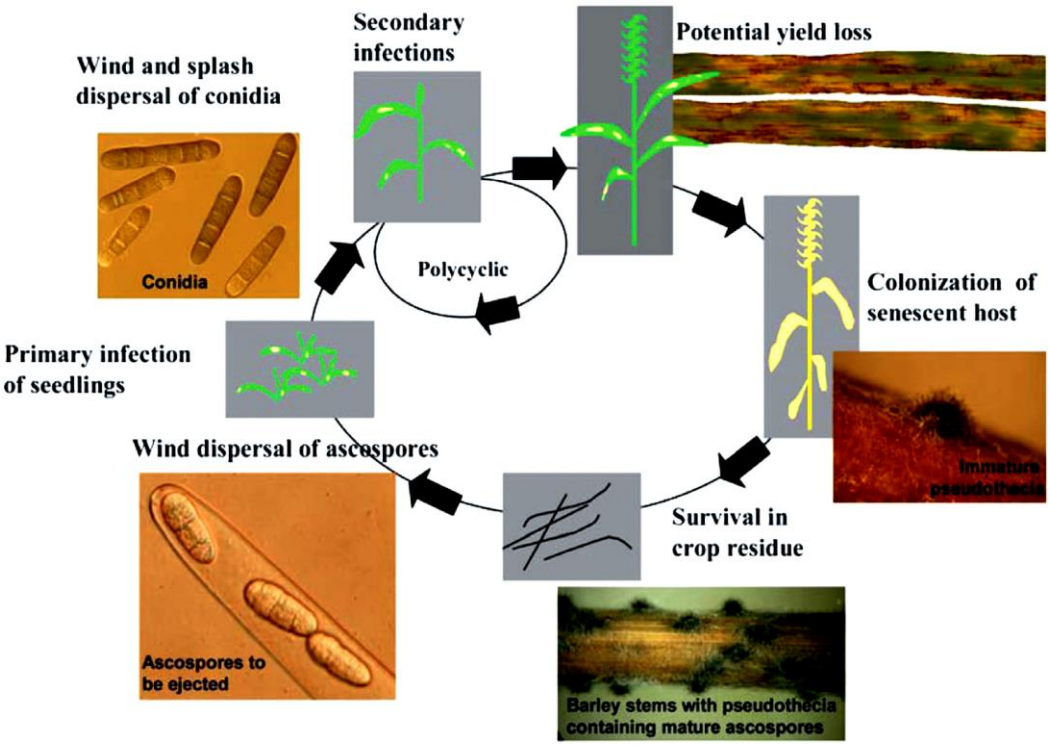


Fig. 1.5 Life cycle of *Pyrenophora teres* (Liu *et al.*, 2011)

During asexual reproduction, *P. teres* produces conidia that may arise in a single form or in groups of two or three on top of conidiophores (Liu *et al.*, 2011; Mathre, 1997; McLean *et al.*, 2009). Conidia are smooth, cylindrical sub-hyaline to yellowish brown with 4-6 pseudosepta (Fig. 1.6) (Liu *et al.*, 2011; Mathre, 1997; McLean *et al.*, 2009) .

Fig. 1.6 *Pyrenophora teres* conidium (asexual spore) (scale bar 40µm)

Unlike ascospores, conidia are not actively discharged, but are released by strong air currents or water splash (Kenneth, 1964). Thus, conidia can be carried longer distances either by strong winds or by rain; causing further secondary infections in upper leaves and neighbouring fields (Jordan, 1981).

Sexual reproduction of *P. teres*, which occurs randomly under certain conditions, will lead to a genetically diverse progeny (Campbell *et al.*, 2002; Kenneth, 1962; Lehmensiek *et al.*, 2010; Rau *et al.*, 2003; Shipton *et al.*, 1973). As with all heterothallic ascomycetes, *P. teres* has a single locus, two-allele mating system, which limits the occurrence of sexual reproduction to opposite-type crossing (Kronstad & Staben, 1997). As such, the sexual cycle can only be developed through the interaction of two fungal strains of different mating-types (MAT) idiomorphs; *MAT 1-1-1* and *MAT 1-2-1* (Kronstad & Staben, 1997; Rau *et al.*, 2007; Turgeon & Yoder, 2000).

Sexual reproduction plays a vital role in shaping the genetic population structure within the two forms of the pathogen. Sexual reproduction between the two forms is a rare event (Rau *et al.*, 2007), however some studies have suggested that recombination between *Ptt* and *Ptm* isolates has occurred in the field (Campbell *et al.*, 1999 ; Campbell *et al.*, 2002; Crous *et al.*, 1995; McLean *et al.*, 2014). In these studies, unique net- and spot-type DNA bands were identified in field collected isolates using Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers.

Sexual reproduction can take place under suitable conditions in the laboratory (Afanasenko *et al.*, 2007; McDonald, 1963; Peever & Milgroom, 1992; Smedegård-Petersen, 1971; Weiland *et al.*, 1999) resulting in mature pseudothecia that contain ascospores, which are in the case of crosses between forms, hybrids. There are three main factors that are crucial to the pseudothecial development; moisture, temperature and light (Odvody *et al.*, 1982). Even though both: temperature (15-18°C) and high humidity are necessary for the ascospores' maturity, light is as important for the initiation of pseudothecia (Friesen *et al.*, 2003; Pfender & Wootke, 1987; Summerell & Burgess, 1988).

Hybrids produced from crossing net and spot form isolates in the laboratory expressed a wide range of symptoms; either net-type or spot-type symptoms or intermediate symptoms (Crous *et al.*, 1995). Hybrids were also tested for fungicide sensitivity. Results indicated that hybrids expressing intermediate

(non-differential) symptoms were resistant to all the effective fungicides even those fungicides proven to be effective on either of the parental isolates (Campbell *et al.*, 1999) indicating the emergence of new virulences that are resistant to all fungicides trialled. Furthermore, hybrids of SFNB x NFNB were able to maintain their virulence and fertility (Campbell & Crous, 2003).

1.2.3 Infection process

Once conidia or ascospores land on the surface of a barley leaf, germination is initiated (Kenneth, 1962; Shipton *et al.*, 1973). In the presence of moisture and favourable temperature, germination can start within a few hours (Shipton *et al.*, 1973). Infection starts with the direct penetration of the leaf, resulting in small pinpoint lesions expressed within 24 hours (Atanasoff, 1920). Infection normally takes place between five and more than 30 hours after initial inoculation (Caeseele & Grumbles, 1979). During the early stages of the germination of conidia and/or ascospores germ tubes appear above the cuticle of the epidermal cells of the host which later develop into hyphae (Caeseele & Grumbles, 1979). Swollen club-shaped appressoria are formed after the appearance of hyphae. The appressoria penetrate the cellular cuticle and epidermal cells through the pressure of the appressoria and the hydrolytic activity of the pathogen (Caeseele & Grumbles, 1979; Jørgensen *et al.*, 1998). This leads to the immediate death of the penetrated cells indicated by symptoms of necrosis (Lightfoot & Able, 2010). As such, the presence of appressoria is considered to be a key to the pathogenicity of *P. teres* in barley (Ruiz-Roldán *et al.*, 2001).

1.2.4 Symptoms and disease development

Symptom expression is dependent mainly on three factors: host genotype, pathogen virulence and environmental conditions (Liu *et al.*, 2011). Net blotch is expressed first as small circular/elliptical lesions that develop into large dark brown blotches; development of these blotches differs according to the three factors stated above. In susceptible varieties, necrosis appears after chlorotic areas start to appear (Scott, 1991; Steffenson & Webster, 1992). In case of severe infections, whole leaves will have a dry appearance

by anthesis time due to the complete necrosis of the leaves and this may lead to complete death of the plant (Mathre, 1997) (Schaller, 1955).

The two forms of *P. teres* are distinguished according to the symptoms they express on the host rather than their morphology. Even though the initial symptoms of both forms are similar, the developed symptoms are typically distinguishing (Sarpeleh *et al.*, 2007). NFNB produces elongated light brown stripes or lesions on leaves and leaf sheaths of older plants and gives a characteristic netting pattern in juvenile leaves with dark brown necrotic reticulations (Figs. 1.7a & 1.7b). SFNB produces dark brown, round to elliptical spots on leaves and leaf sheaths that are often surrounded by yellowing or distinct chlorotic areas (Figs. 1.8a & 1.8b) (Campbell *et al.*, 1999 ; Rau *et al.*, 2007).

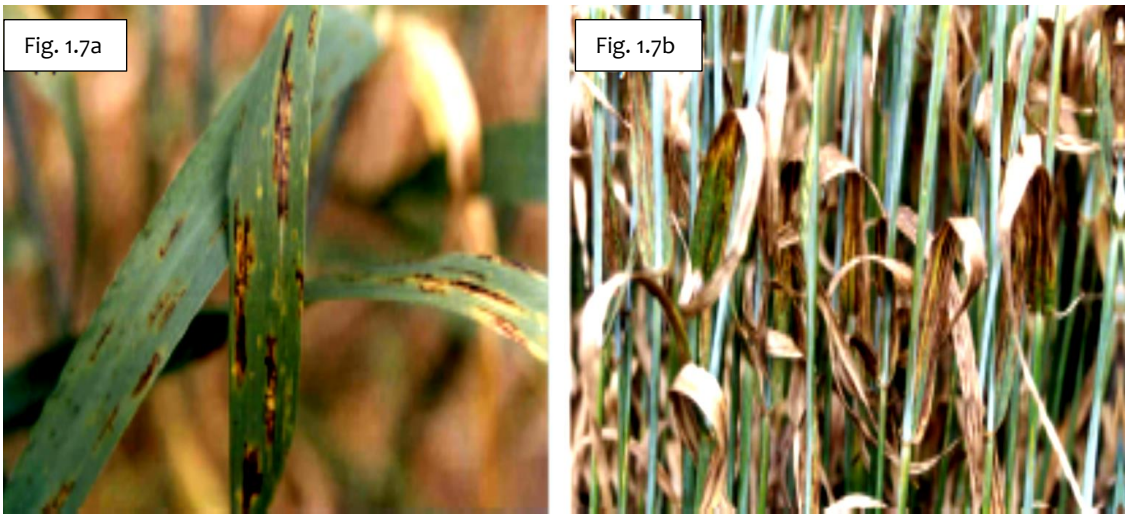


Fig. 1.7a and b NFNB symptoms caused by *Pyrenophora teres* f. *teres (McLean et al., 2009)*

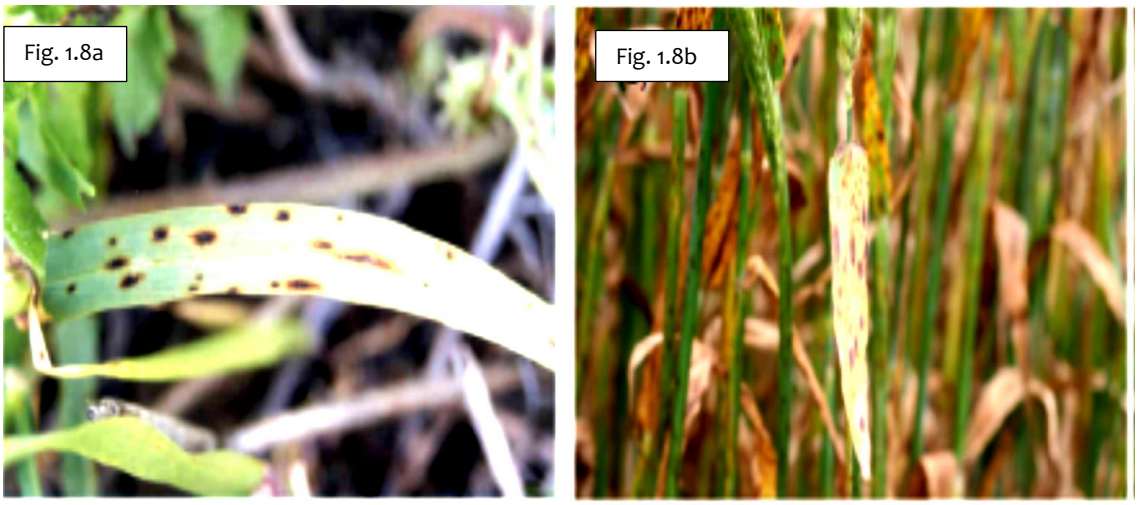


Fig. 1.8 a and b SFNB symptoms caused by *Pyrenophora teres* f. *maculata (McLean et al., 2009)*

Both forms of *P. teres* induce necrosis of the leaf tissues within 24 hours of inoculation mostly due to proteinaceous toxins (Sarpeleh *et al.*, 2009; Sarpeleh *et al.*, 2007). This is then followed by appearance of chlorotic water soaked areas around necrotic lesions (Smedegård-Petersen, 1977). Chlorotic areas were found to contain no hyphal growth, which indicates that cell death is caused by aspergillosamine derived toxins produced by *P. teres* within the host (Smedegård-Petersen, 1977).

Three different toxins have been identified in both forms of *P. teres* (Bach *et al.*, 1979). All three phytotoxic compounds have chemical structures similar to aspergillomarasmine A (Bach *et al.*, 1979). However, the three toxins – named A, B (Smedegård-Petersen, 1977) and C differ in their ability to cause infection to the host (Weiergang *et al.*, 2002). Toxin B (anhydroaspergillomarasmine A) is considered the weakest toxin. Toxin C (aspergillomarasmine A) is the most potent of the three as it causes severe necrosis and light yellow chlorosis areas surrounding this necrosis, while toxin A [L, L-Ɲ-(2-amino-2-carboxyethyl) aspartic acid] is regarded as the second most toxic as it only results in dark chlorotic areas with no necrosis (Weiergang *et al.*, 2002). Interestingly, toxin C is converted into toxin B upon the lowering of the pH of the culture, while toxin A is actually a direct precursor of toxin C production (Friis *et al.*, 1991). Toxins are not specific to certain virulences or isolates of *P. teres* which suggests the absence of differences in barley genotype sensitivity (Sarpeleh *et al.*, 2009) albeit the severity of symptoms are linked to the amount of toxins produced (Sarpeleh *et al.*, 2009; Smedegård-Petersen, 1971) along with the fungal biomass inside the host (Leisova *et al.*, 2006).

Sporulation takes place one week after initial infection (Keon & Hargreaves, 1983); (Smedegård-Petersen, 1977). Sub-hyaline conidial groups of two or three arise either from in between epidermal cells or from stomata though it is not as common (Kenneth, 1962; Lightfoot & Able, 2010). Each of those conidia can sporulate independently within two hours (Kenneth, 1962).

Van den Berg and Rossnagel (1990b) suggested that *Ptm* is capable of infecting barley in less time than that needed for *Ptt.* This is probably due to

the higher quantities of toxins produced by *Ptm* in comparison to *Ptt* (Sarpeleh *et al.*, 2009). Yet, after infection occurs, *Ptm* appears to have a slower rate of growth though clear symptoms of disease appear similarly for both types; 72 h and 96 h for susceptible and resistant lines, respectively (Lightfoot & Able, 2010).

According to Lightfoot and Able (2010), the differences in growth between *Ptt* and *Ptm* are due to differences in the formation of intracellular vesicles, the amount of mycelia developed prior to penetration, the amount of mycelial growth in the mesophyll and the closeness between the collapsed host cells and the fungus. Lightfoot and Able (2010) found that Ptt germinate faster than Ptm as germination initiates after 6h of inoculation for Ptt and after 8h for Ptm. They also found that despite Ptm producing more intracellular vesicles, Ptt shows more extensive mycelial growth.

Around 24h after inoculation, appressoria start to form and the hyphae branch and thicken until the penetrating pegs appear 48h from the inoculation. At this stage *Ptt* shows more spread in the mesophyll. Host cells that are closest to the fungus would brown after 72h from inoculation, leading to the appearance of necrotic spots which develop with further hyphal branching and consequently wider cell browning by 120h. By 168h, clear chlorosis and necrosis symptoms appear due to the rise of conidiophores out of the cuticle and total senescence of host cells (Lightfoot & Able, 2010).

1.2.5 Disease management

1.2.5.1 Cultural and mechanical management

Other controlling methods include crop rotation and stubble destruction to eradicate the source of primary infection (McLean *et al.*, 2009). To prevent the emergence of new infections, all the crop stubble should be removed, buried and burned (Jordan & Allen, 1984) .

It is recommended to grow non-host crops after each barley growing season. Pulses and oilseeds can be grown for this purpose and such rotation should take three years (Bretag, 2009).

1.2.5.2 Biological control

Johnsson *et al.* (1998) applied liquid bacterial culture of *Pseudomonas chlororaphis* over barley seeds infected with *P. teres* in a five year trial in Sweden. Based on this trial, Johnsson *et al.* (1998) found out that *P. chlororaphis*, strain MA 342 can be used as a disease suppressor with consistent effect. However, when compared to using fungicides for disease management, this method is not as efficient (Johnsson *et al.*, 1998).

1.2.5.3 Fungicides

A widely spreading method of disease management is applying chemical control either as seed treatment to reduce the primary inoculum of the disease (Liu *et al.*, 2011) or as foliar fungicide (McLean *et al.*, 2009). Foliar fungicide application is uneconomic since multiple applications are necessary (Liu *et al.*, 2011; McLean *et al.*, 2009; Shipton, 1966). According to van den Berg and Rossnagel (1990a) a significant decrease in SFNB severity depends on the active ingredient of the fungicide used and its rate, number of applications of the fungicides and their timings, and the development of pathogen's resistance to the fungicide. Khan (1989) has intensively investigated the application of foliar fungicide for controlling SFNB in Western Australia and concluded that there is a strong correlation between the number of times the fungicide is applied and the severity of the disease. In his study, Khan (1989) reported that with a single application of foliar fungicide at 99% disease severity or infection, a grain yield increase of 23% is achieved. This grain yield increase can raise up to 41% if the fungicide is applied twice (Khan, 1989).

Due to the relatively high cost of repetitive applications of foliar fungicides studies have been carried out to investigate the probability of minimizing the number of applications. As a result, one strategy that depends on the early application of fungicide has been adopted for some time now even though it needs further evaluation. In this strategy, the fungicide is applied early at a predetermined stage to protect the seedling from infection. Applying this method can increase the yield by 72% (Gallagher *et al.*, 1975; McLean *et al.*, 2009; Paveley *et al.*, 2000).

Jayasena *et al*. (2002) carried out experiments to evaluate ten different fungicides. Out of the tested fungicides pyraclostrobin, propiconazole, epoxiconazole and a mixture of propiconazole with iprodione showed effectiveness in controlling SFNB, yield increase and enhanced grain quality upon single application. However, not all of these chemicals are registered in Australia. Instead, β-methoxyacrylic fungicidal derivatives such as strobilurins which are known for inhibit mitochondrial activity of *P. teres* are currently used for chemical control of the disease (Bartlett *et al.*, 2002; McLean, 2011). For seed treatment, Dividend® is the only treatment registered in Australia (Bretag, 2009).

1.2.5.4 Host resistance

To develop a disease resistant barley variety, parallel breeding projects on barley are required as they incorporate multiple disease resistances into one variety. For example most prominent pathogens for all the varieties of *Hordeum vulgare* are tested rather than one pathogen only. For instance; between 1980 and 1986 a project of developing a barley line resistant to spot blotch disease, caused by *Bipolaris sorokiniana*, succeeded in developing two lines: Morex and Robust, which were then used intensively in Minnesota (Wilcoxson *et al.*, 1990). However, those two lines turned out to be susceptible to the net blotch disease caused by *P. teres*, which resulted in great yield losses (Wilcoxson *et al.*, 1992). These days in Australia, such issues are avoided by Projects such as the National Variety Trials (NVT) that take place all over Australia. The National Variety Trials (NVT) project was initiated by the Grains Research and Development Corporation (GRDC) and managed by the Australian Crop Accreditation System Limited (ACAS) since 2005. Barley is one of many cereals tested through the NVT. However, not all the lines of barley are tested, only the lines that are nominated by barley breeders to ensure the lines are as close as possible to commercial release (Hochman *et al.*, 2012; Park, 2008). Those nominated lines are then evaluated according to Minimum Disease Standards (MDS), which are a set of characters that provide minimum disease protection to the lines released (Wallwork, 2007).

Even though the resistance of the host to the disease is isolate-specific (Afanasenko *et al.*, 2007), the host-pathogen interaction is dependent on many factors (Afanasenko *et al.*, 2007), such as the environmental conditions, nutritional conditions of the host and pathogen and the development of each individual organism – ontogenetic factors (Steffenson & Webster, 1992) .

The necessity of identifying specific avirulence gene(s) in the pathogen and specific resistant gene(s) in the host arises from the high variability of *P. teres* and its ability to produce new virulences (Steffenson & Webster, 1992; Tekauz, 1990), along with the frequent lack of barley lines resistant to all the virulences present in the local environment (König *et al.*, 2014).

Isolates collected from Europe, Canada, Australia and USA, have shown that disease resistance is isolate-specific (Afanasenko *et al.*, 2007). It was Schaller (1955) who first reported the presence of a single dominant gene (*Pt1*) in Tifang barley, responsible for expressing resistance to net blotch. This report was later followed by the discovery of further genes responsible for Tifang resistance to net blotch (Ho *et al.*, 1996). These genes are *Pt2*, which is tightly linked to *Pt1*, *Pt3 (Mode & Schaller, 1958)*, *Pta* – also present in Ming and CI 2330, and *Rpt3d (Bockelman et al., 1977)*. Another two genes; *Rpt1b*, located on chromosome 3H and *Rpt2c*, located on chromosome 1H, were found to be responsible for the resistance to net blotch in the barley variety CI 9819 (Bockelman *et al.*, 1977; Khan & Boyd, 1969). Some barley cultivars such as “Ming” and “Harbin” were found to possess at least one gene that is responsible for resistance to *P. teres* f. *teres* (Weiland *et al.*, 1999).

Wilcoxson *et al.* (1992) mentioned that the reaction between pathogen and host is similar with either adult or juvenile plants. This was confirmed by more than one study (Cakir *et al.*, 2003; Grewal *et al.*, 2008) where the 6H locus responsible for the resistance to net blotch was found to be effective in both the seedlings and adult plants.

Other studies have been conducted to detect more durable resistance traits in barley than the resistance that single-gene(s) might show. Those studies

have eventually led to identifying quantitative resistance loci (QTL) on the barley chromosomes 1H, 3H, 6H and 7H. An early study to detect QTL regions associated with resistance to net blotch in barley was conducted by Steffenson *et al*. (1996) who used a DH population of barley Steptoe/Morex. That study revealed two QTL regions, a major one on 6HL and another minor one on 6HS (Steffenson *et al.*, 1996).

A QTL on chromosome 6H was also reported by Manninen *et al.* (2000) who used a cross between resistant and susceptible barley lines, CI9819 and Rolfi, respectively. The QTL region reported in the study of Manninen *et al.* (2000) might correspond to the 6HL QTL reported by Steffenson *et al* (1996) (Ma *et al.*, 2004). Numerous other studies have identified a major QTL for NFNB resistance on chromosome 6H (Abu Qamar *et al.*, 2008; Emebiri *et al.*, 2005; Friesen *et al.*, 2006).

Another study carried out by Cakir (2003) using two doubled haploid (DH) barley crosses derived from Tallon/Kaputar (TK) and VB9524/ ND11231 (VN) was conducted, in which Cakir *et al.* (2003) confirmed the association between QTLs on chromosomes 3H and 6H and the resistance to NFNB. Moreover, they have also detected other QTL regions for resistance on chromosome 2H.

Ma *et al*. (2004) produced a DH population between two barley lines of different reactions to *Ptt,* Chevron and Stander. Their study revealed the presence of one resistance gene (*Rpt*) on chromosome 6H in line Chevron and a resistance QTL region on chromosome 2H in Stander (Ma *et al.*, 2004).

Grewal *et al.* (2008) identified QTLs on chromosomes 2H, 4H and 5H, besides a major QTL region on chromosome 6H (Grewal *et al.*, 2008).

Gupta *et al.* (2010a), have also identified the resistance regions in lines Pompadour and Stirling. Gupta *et al.* (2010a) developed a barley population of 200 double haploid lines (DHL) by crossing barley multi-resistant line Pompadour with another wide-spread line: Stirling. Genetic linkage analysis and bulked segregant analysis (BSA) showed two major NFNB resistance

QTL regions on chromosomes 3H and 6H of the parent Pompadour to *Ptt* isolates 97NB1, 95NB100 and NB81. As to Stirling, a resistance QTL region on chromosome 6H was also detected but not precisely identified either as a close region to that QTL on Pompadour's 6H chromosome or an allele to it. Besides the 6H QTL a QTL was also identified on chromosome 3H with a minor effect on the F1 lines (Gupta *et al.*, 2010a). Despite the overall variation resulting from different allele-combinations between lines Pompadour and Stirling, QTL regions on chromosomes 3H and 6H still show isolate-specific interactions (Gupta *et al.*, 2010a; Manninen *et al.*, 2006).

As to the resistance to *Ptm* (SFNB), Friesen *et al.* (2006) identified a QTL region for SFNB resistance on chromosome 4H, while Grewal *et al.* (2008) have also identified a major QTL region on the same chromosome besides another one on 7H (Friesen *et al.*, 2006; Grewal *et al.*, 2008).

An association mapping study was conducted by Wang *et al*. (2015) using 898 elite barley lines. Interestingly, that study has revealed 29 QTL regions associated with resistance of barley to SFNB in each of the seven chromosomes of barley. Each of those QTLs detected were linked to resistance in both the seedling and adult stages (Wang *et al.*, 2015).

1.2.6 Genetics of pathogen avirulence/virulence

As a heterothallic haploid fungus, *P. teres* is a highly diverse pathogen (Shipton *et al.*, 1973). This diverse nature makes it challenging to develop efficient and long lasting methods for disease management as many of the progeny resulting from sexual reproduction have been shown to induce intermediate reactions that were not similar to the reactions of the parents (Weiland *et al.*, 1999).

As a general rule in plant pathogens, avirulence genes (*Avr*) of the pathogen are the gene(s) responsible for encoding antigenic molecules that are recognized by a matching receptor in the host causing a hypersensitivity response (Hammond-Kosack & Jones, 1996). Those *Avr* genes are considered modifier genes or epistatic genes to the pathogenic (*Path*) ones, since in their presence, *Path'*s products are not able to induce disease inside

the host (De Wit, 1995; De Wit, 1997) and thus the presence of those *Avr* genes is enough to supress the virulence or pathogenicity of the pathogen in spite of the presence of the *Path* genes.

Weiland *et al.* (1999) investigated the inheritance of virulence within the 82 progeny resulting from a cross between the two *P. teres* f. *teres* isolates: 15A (avirulent) and 0-1 (virulent). The progeny were phenotyped across Harbin. After extracting DNA from each of the *P. teres* isolates, it was subjected to the random amplified polymorphic DNA (RAPD) technique and Bulked Segregant Analysis (BSA) to identify molecular genetic markers associated with the virulence phenotype. Five of the 86 RAPD markers used were found to be associated with low virulence. Three of these makers were co-segregating with each other and were located 3.8 cM from the avirulence gene, *avrHar* (Weiland *et al.*, 1999). Out of the 82 progeny of the 15A × 0-1 cross, 40 individuals showed low virulence while the other 42 showed high virulence. That is a near 1:1 segregation of high to low virulence to the cultivar “Harbin” which complies with the nature of *P. teres* as a haploid fungus as it also gives a strong implication that a single major gene is probably controlling the virulence of *Ptt* on Harbin .

Some studies have shown an indirect relationship between the pathogenicity of *P. teres* and the expression of particular genes. Upon investigating the genes expressed during the conidial germination process of *P. teres*, Dilger *et al.* (2003) found that the expression of many genes involved in signal transduction and gene regulation increased during the early stages of spore germination (Dilger *et al.*, 2003).

Vergara *et al.* (2003), found that the transcription of a specific cDNA fragment is induced in the pathogen in the presence of the barley leaf cell wall after *P. teres* f. *teres* infection. The sequence of this fragment was homologous to many genes that are coding regulatory proteins. This suggests that this cDNA fragment is possibly involved in the differential regulation of *P. teres* pathogenicity (Vergara *et al.*, 2003).

Mironenko *et al.* (2005) suggest that the formation of new virulences of *P. teres* might be due to more than one factor: the suppressor genes that are

involved in meiotic recombination, the recombination of avirulence genes and the mutation within the *P. teres* population. However, it is not totally accurate to apply results from one study on certain isolate(s)/cultivar(s) interactions to other isolate(s)/cultivar(s), since virulences differ among different pathotypes. Those pathotypes may also differ depending on their geographical origins (Ho *et al.*, 1996; Steffenson & Webster, 1992).

In order to improve management strategies it is important that we understand more about the interactions between the host and the pathogen. To investigate the pathogen side of this interaction the characterisation of the virulence/avirulence genes is highly desirable. To do this it is vital that a complete genetic map of this pathogen is developed. This can then be used in QTL analysis to identify genomic regions associated with virulence. Using the same isolates of *P. teres* for crossing as in the Weiland *et al.* study (1999): 15A and 0-1, Lai *et al.* (2007) constructed an AFLP-based linkage map. However, this cross was used to evaluate the genetics of avirulence associated with three different barley lines: Prato, Tifang and Canadian Lake Shore (CLS) instead of Harbin. After the assembly of 16 linkage groups out of the 116 mapped markers, it was found that two main genes are responsible for the avirulence of *P. teres* to Prato: *AvrPra1* and *AvrPra2* (Lai *et al.*, 2007). On the other hand, Beattie *et al.* (2007) chose a collection of 10 net form (*Ptt*) and spot form (*Ptm*) isolates to be screened across a set of eight barley lines. This was then followed by mating one avirulent isolate: WRS 1906 with a highly virulent isolate: WRS 1607. The progeny were then phenotyped for reaction on one barley variety: Heartland. Six amplified fragment length polymorphism markers closely linked to the avirulence gene ($Avr_{Heartland}$) were then identified following a bulked segregant analysis. This study demonstrated that the *P. teres*–barley pathosystem follows the gene-for-gene model and is the first step toward map-based cloning of this gene (Beattie *et al.*, 2007).

1.2.7 Simple Sequence Repeat Markers (SSRs):

Simple sequence repeat markers (SSRs) are a group of repetitive DNA sequences that represent a significant portion of a genome and they range

between mono to penta-nucleotide units (Davierwala *et al.*, 2000; Hamada *et al.*, 1982; Powell *et al.*, 1996). SSR markers are polymerase chain reaction (PCR) based markers that are mainly used to detect polymorphisms in a genome and they were first reported based on human and mammalian biology (Hamada *et al.*, 1982). However, it was Powell *et al.* (1996) who first reported SSRs use in plants. Since then, SSR markers have been widely used in plants due to their advantages. Besides being highly informative and reproducible, SSRs can be easily detected by PCR and displayed by gel electrophoresis, they are also of multi-allelic nature and can be transferred between populations and need only small amounts of DNA (Collard *et al.*, 2005; Jones *et al.*, 1997; McCouch *et al.*, 1997; Powell *et al.*, 1996).

Many different researcher groups have produced SSR primer sequences for studying the barley genome (Becker & Heun, 1995; Liu *et al.*, 1996; Ramsay *et al.*, 2000). Such resources were later used to identify the regions of resistance to net blotch in barley (Gupta *et al.*, 2011a; Gupta *et al.*, 2010b; König *et al.*, 2013; König *et al.*, 2014). However, there is barely any work published for using SSRs to identify virulence genes on *P. teres* itself, except for a recent research conducted by Shjerve *et al.* (2014) who screened a cross between American Ptt isolates 15A and 6A on barley lines Rika and Kombar, which were tested previously by the same group for resistant QTL regions (Abu Qamar *et al.*, 2008). The mentioned study revealed four virulence QTL regions on *Ptt*, VK1 and VK2, virulent to Kombar and VR1 and VR2, virulent to Rika (Shjerve *et al.*, 2014).

1.2.8 High Resolution Melt Assay - HRM™

High Resolution Melt (HRM) analysis is a post-PCR analysis that characterizes the dissociation behaviour of each of the DNA sequences tested. The DNA samples loaded are mixed with a double-stranded DNA (dsDNA) intercalating fluorescent dye that cannot intercalate with single-stranded DNA (ssDNA). Each dsDNA will turn into an ssDNA at a specific temperature (T_M). The fluorescence of the dye diminishes gradually until it totally vanishes at the melting temperature (T_M) signalling the

temperature/behaviour of that specific sample of DNA sequence (Fig. 1.9) (White & Potts, 2006).

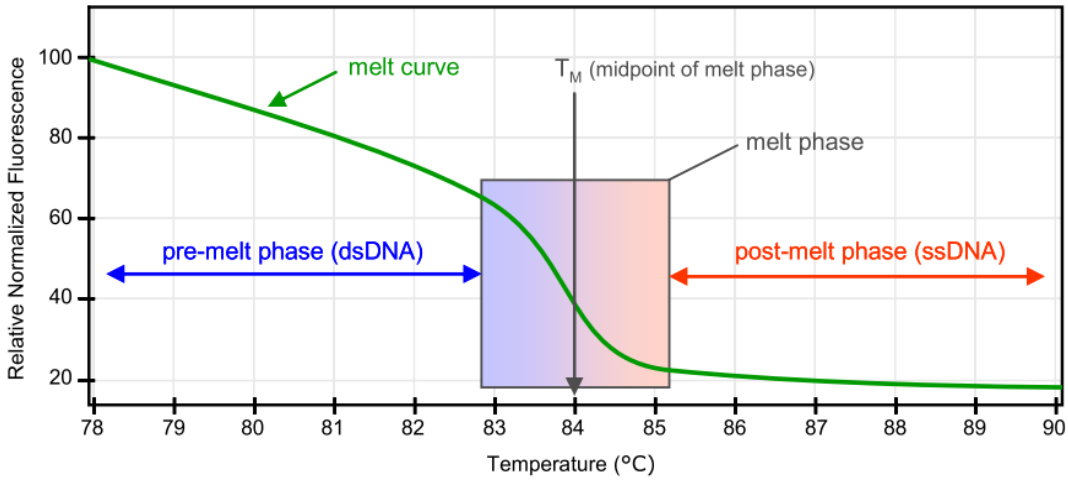


Figure 1.9: Fundamentals of a typical HRM (high resolution melt) plot (Science, 2006).

The HRM technique can be used for many applications, including the comparison between genotypic and phenotypic data, identifying species, determining the occurrence of allele within a population, detecting heterozygosity, detecting mutations, DNA mapping and DNA fingerprinting (Science, 2006; White & Potts, 2006).

Lehmensiek *et al.* (2008) were the first to report the use of HRM to identify and map Single Nucleotide Polymorphism (SNP) markers in barley. In their study on covered smut of barley, caused by *Ustilago hordei*, Lehmensiek *et al.* (2008) fine-mapped the barley genome and identified two Expressed Sequence Tag (EST) markers, AV836787 and CK123008 that are closely linked to *Ruh.7H,* a major single gene on barley, responsible for resistance to covered smut in cultivar Sloop.

1.2.9 Diversity Arrays Technology (DArT®):

For whole genome genotyping, methods such as SNP chips are available. Diversity Arrays Technology (DArT®); http://www.diversityarrays.com, however, is the most recently used methods for genetic mapping. DArT® technology is a cost-effective technology that is based on microarray hybridization, which detects the presence/absence of individual DNA fragments (Grewal *et al.*, 2008; Jaccoud *et al.*, 2001; König *et al.*, 2014). A

DArT-seq™ map can be developed by combining the DArT markers and Next-Generation Sequencing (NGS) platforms (Kilian *et al.*, 2012; Sansaloni *et al.*, 2011). DArT markers have a wide range of uses in the field of genetic diversity assessment (Cruz *et al.*, 2013). Using a high-throughput SNP genotyping method, markers' density and resources have increased significantly, which along with the DArT-seq™ mapping led to the construction of high-density consensus map of many crops, including barley (Wenzl *et al.*, 2006).

1.3 Phenotyping methods

Despite the increased use of molecular markers and other advanced molecular techniques in the breeding process, it is impossible to obtain efficient results from techniques such as QTL analysis and association mapping unless they are applied in conjunction with accurate phenotypic data. Phenotypic assays generally depend on the visualization of the disease by scoring the symptoms according to a scale that assesses severity. This occurs by inoculating each host genotype with a standard level of inoculum of the pathogen, followed by incubation in conditions favourable for disease development and then assessing the disease after a set period of time. A number of phenotyping assays have been developed for screening net blotch disease.

1.3.1 Seedling assay

Most widely and frequently used phenotyping assay is the seedling assay. This method involves spraying a conidial suspension onto 10-14 day old seedlings. A "Standard Inoculation Technique" developed by Khan and Boyd (1969) is usually used for this method. Generally, a conidial suspension is used for the inoculation. This is prepared by adding distilled water to the culture plates to dislodge the mycelium. This suspension, after spore counting and dilution to a set spore concentration, is then used for spraying the seedlings. Inoculated seedlings are then exposed to 95-100% humidity under reduced light for 48 hours at 18-24°C before being transferred to the glasshouse at standard temperature (Khan & Boyd, 1969). However, exposure to humidity can reduce the time needed for initial non-differentiating

disease expression to 24 hours (Louw *et al.*, 1994; Weiland *et al.*, 1999). Seedlings are scored seven to fourteen days after inoculation (Crous *et al.*, 1995; Khan & Boyd, 1969; Louw *et al.*, 1994; Weiland *et al.*, 1999). Symptom assessment is slightly different between the two forms of the disease. For NFNB, a scale of 1-10 is used whereas for SFNB a non-continuous 1-9 scale is used (Tekauz, 1985). The SFNB scale according to Tekauz (1985) shows the reactions 1-3, 5 and 7-9 with the absence of reactions 4 and 6. Responses 1-4 for the NFNB and 1-3 for SFNB are considered indicators of the pathogen avirulence or the resistance of the cultivar, while reactions 4-5 for the NFNB and 5 for SFNB indicate moderate virulence and/or susceptibility of the pathogen and the cultivar, respectively. Reactions 6-10 for NFNB and 7-9 in case of SFNB indicate the virulence of the pathogen or susceptibility of the inoculated cultivar (Tekauz, 1985).

However, this method presents a biosecurity risk when novel isolates generated in the laboratory are being screened as there is a high potential that these isolates/hybrids carry new virulence that have not been investigated before.

1.3.2 Detached leaf assay (DLA) – Droplet method

Another method for phenotyping net blotch disease of barley is the “detached leaf assay” (DLA) (Fig. 1.10) (Afanasenko *et al.*, 2007; Steffenson & Webster, 1992). DLA was developed to substitute the usual seedling test or to be used as a pre-field trial.

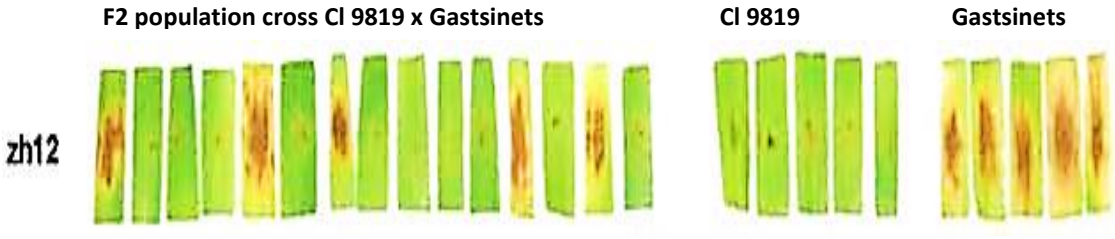


Fig. 1.10 Each of the first 15 leaf-segments in the vertical column from the left represents a single progeny of the Cl 9819 x Gastsinets barley cross tested with *Pyrenophora teres* isolate (zh12) (Afanasenko *et al.*, 2007)

Field trials used for breeding resistant varieties are very costly and require multiple trial sites and years to identify the interactions between the host, the pathogen and the environment. The DLA method is less expensive and time consuming than field trials and an effective method for screening the disease (Kumar *et al.*, 2011). Another advantage of using the DLA method is that it needs a relatively small space, but more crucially, it allows full control of the conditions (Kumar *et al.*, 2011) and complete confinement of the tested isolates, which is a key requirement in case the isolates tested are laboratory-generated recombinants with virulences potentially different to those of the parents.

1.3.2.1 DLA correlation with correspondent field trials and seedling assays

Kumar *et al.* (2011) evaluated the DLA method by using barley genotypes of known reactions to *Fusarium* head blight (FHB). To do this, they compared the DLA method to another standard method, the field trial. Three partial disease resistance (PDR) criteria were used for this evaluation, latent period (LP), lesion size (LS) and macroconidial counts (MC). Three tests were carried out where each criterion was tested using the both methods, the field trials and the DLA. One of the three tests showed negative correlation between the incubation period and the field rating, one test showed positive correlation between the lesion size in the DLA and the field disease severity, while two tests out of the three have shown negative correlation between the latent period and the field rates. In general, the lesion size in field test is smaller than its correspondent in the DLA, yet, both methods are highly correlated (Brooks, 2008). The physiological state of the detached leaf is crucial to its interaction with the pathogen; the more mature the leaf is, the more resistance it has against the disease (Péros *et al.*, 2006).

1.3.3 Phytotoxin assay

Another phenotyping method is the phytotoxin assay. For the phytotoxin assay method the toxins are produced in vitro where the *P. teres* culture is partially purified pressed and centrifuged based on Smedegård (1977) to isolate the protein molecules of the toxin (Sharma, 1984).

Smedegård-Peterson (1977) isolated pure toxins – A and B - from *P. teres* sterile filtrates. Despite being extracted from cell-free cultures, these toxins demonstrated symptoms of net blotch upon inoculating them on healthy barley leaves (Smedegård-Petersen, 1977). This method was later on used as a phenotyping assay (Phytotoxin assay) (Ismail *et al.*, 2014; Sarpeleh *et al.*, 2009; Sharma, 1984; Yoder, 1983). Those studies suggest that the phytotoxin assay may replace the initial screening of *P. teres* in barley lines during the initial stages of resistant barley breeding programmes especially with the availability of purified phytotoxins (Weiergang *et al.*, 2002).

Sharma (1984) has assessed the reaction of 21 spring barley cultivars to *P. teres* via three different methods; whole seedlings, detached leaf assay (DLA) and phytotoxin assay. All three methods showed strong correlation coefficients: 0.88 between the seedling assay and the DLA, 0.83 between the DLA and the phytotoxin assay and 0.91 between the seedling assay and the phytotoxin assay (Sharma 1984). Interestingly, DLA results using young plants have shown good correlation to the seedling test even though the latter was performed using much older plants. Although the overall correlation between the seedling test and the phytotoxin assay was high, the phytotoxin assay could only indicate the most resistant and most susceptible cultivars rather than intermediate ones (Sharma 1984).

Another issue regarding the phytotoxin assay is that using a crude filtrate of the toxins may produce further symptoms that are irrelevant to the pathogenicity of *P. teres*, due to the presence of secondary metabolites in such filtrates. Thus, pure toxins are recommended for this test (Yoder, 1983).

1.4 Summary

Despite its devastating impact on the industry and numerous studies, some aspects of net blotch of barley need to be further investigated. There are already some phenotyping assays that have been used for screening net blotch among other plant diseases, but none of them can confine a highly diverse pathogen like *P. teres* while screening it accurately. For instance, field trials and seedling assay are the most widely used methods, but they are laborious and require considerable space besides that it is hazardous to

use them for screening hybrids/recombinants of *P. teres* as this would mean the release of potentially new virulences to the environment. On the other hand, the phytotoxin assay and DLA represent a more confining alternative as all isolates are screened in the laboratory. Yet, the phytotoxin assay is technically demanding since pure toxins need to be tested to exclude any irrelevant symptoms induced by metabolites if a crude filtrate is used. That leaves us with DLA-droplet method as an approachable phenotyping method. However, with using small segments of barley leaves, an indication of the symptoms type (NFNB or SFNB) upon screening hybrids would not be displayed. That is why a new phenotyping method (DLA – spray) was developed as it is more confining than the seedling assay and field trials, simpler than phytotoxin assay and more indicative than the DLA – droplet method.

Using the DLA – spray method, an investigation was conducted to determine new virulences that may rise from sexually mating *Ptt* x *Ptt* isolates and *Ptt* x *Ptm* isolates. Such knowledge can be added to our understanding of the pathogen. Even though *Ptt* x *Ptm* mating is a rare event, it has been reported more than once reflecting a considerable risk of losing resistant barley varieties should these hybrids produce virulences different to those of the original parents. This leads to the significance of studying hybrids and recombinants of *P. teres* to prevent such a risk.

Finally, looking at the management methods used to control net blotch, it becomes clear that there are severe drawbacks of each method. While biological control still needs intensive evaluation, other environmentally friendly methods such as crop rotation are not economically functional since the farmer is required to have a three years break between each barley harvest. Other methods such as burning the stubble and using fungicides comprise a great hazard to environment and even to human health. This all leave us with one solution which is developing stable resistant barley lines that can endure the diverse nature of the pathogen. To do this, identification of both, resistance QTLs in the barley genome and virulence QTLs on the pathogen's genome is required. As mentioned in the Host Resistance section various studies have been carried out revealing many QTL regions on barley

genome associated with resistance to the disease. However, QTL regions associated with the virulence of *P. teres* were not investigated as much. Consequently, the last aim of our study is to fine map a QTL region associated with virulence in a *Ptt* x *Ptt* cross using SSR markers.

1.5 Aims of the study

1. Identify the best method for phenotyping net blotch disease

Using seedling assay as a standard, both DLA methods, droplet and spray, will be evaluated to support the 1st hypothesis:

DLA – spray method can replace both seedling assay and DLA – droplet method for phenotyping net blotch of barley.

2. Determine virulences of progeny produced by sexual recombination between different isolates of *Pyrenophora teres*

Using progeny of *Ptt* x *Ptt* and *Ptt* x *Ptm* crosses produced in the laboratory, new virulences will be investigated to support the 2nd hypothesis:

Hybrids of P. teres express virulences different to their parents'.

3. Identify and map new virulence genes in *Ptt* x *Ptt* population NB29xNB85

Using SSR markers, an existing map of *P. teres* f. *teres* will be fine mapped to support the 3rd hypothesis:

SSR markers can be used to fine-map QTL region associated with virulence in P. teres f. teres

2 Chapter 2: Evaluating different phenotyping methods

2.1 Introduction

The seedling assay is considered to be the standard method of phenotyping net blotch of barley. However, when studying laboratory produced *P. teres* hybrids with potentially new virulences, it is crucial to confine the pathogen. A DLA – droplet method has previously been published (Afanasenko *et al.*, 2007) which confines the isolates within trays. Thus this method is suitable for screening recombinants of *P. teres.* However, using this method it is difficult to determine whether an isolate has the typical net-form or spot-form lesions. Therefore, the first aim of this study is to develop a method that still confines the isolates but produces virulence reactions similar to those obtained with the conventional seedling assays. To achieve this, the DLA – droplet method has been modified to a spray method. The new method was compared to both the DLA –droplet method and the standard seedling assay.

HYPOTHESIS:

DLA – spray method can replace both seedling assay and DLA – droplet method for phenotyping net blotch of barley.

2.2 Materials and methods

2.2.1 The plant and fungal material

Two NFNB isolates, NB053 and NB085 and two SFNB isolates, SNB171 and SNB74S were used to compare the different phenotyping methods (Table 2.1). These isolates were screened across eight barley varieties that differ in reaction to each of the two forms of net blotch (personal communication Ryan Fowler and Greg Platz, DAFQ; Table 2.2). The data obtained from the DAFQ show the reaction of some barley varieties to NFNB isolates NB053 and NB085 and one SFNB isolate SNB320. No previous data were available for SNB171 and SNB74S and therefore SNB320 was used as an example.

Table 2.1 List of the *Pyrenophora teres* isolates used for phenotyping methods evaluation, with the leaf-symptoms observed, geographic origin, year of collection and host (Lehmensiek *et al.*, 2010).

Isolate	Fungus	Sampling location/state	Town	Year
NB053	PTT	SA	Narracoorte	1994
NB085	PTT	QLD	Gatton	1995
SNB171	PTM	WA	Palinup River	1995
SNB74S	PTM	QLD	Millmerran	1995

Table 2.2 Barley differential set and their reactions with the actual scores to *Pyrenophora teres* isolates, NB053, NB085 and SNB320 (personal communication Ryan Fowler and Greg Platz, DAFQ). The actual scores are in brackets. Scores between 0-3.9 indicate resistance (R), 4-4.9 indicates intermediate reaction (I) and 5-10 indicate susceptibility (S). The exact scores of some varieties are not available, so only the reaction rank: R, I or S is shown.

Barley variety	Reaction to NB053	Reaction to NB085	Reaction to SNB320
CI 5791	R (1)	R (1)	S
Prior	R (2)	S (10)	S
Skiff	S (8)	R (1.6)	I
Keel	Not Available	S (9.6)	R
Westminster	Not Available	Not Available	S (8.5)
Dash	Not Available	Not Available	S
NRB11313	Not Available	Not Available	R (2)
Skipper	Not Available	Not Available	R

Key	
Resistant	
Intermediate	
Susceptible	

2.2.1.1 Planting

For the seedling assay, five seeds each of the eight varieties were planted in a 50mm wide by 120mm deep pots using Searles Premium potting mix and placed randomly in trays in the glass house 19 days prior to the inoculations. For the detached leaf assay – both droplet and spray method, 20 seeds of each variety were planted in a 100mm pot and placed randomly in trays in the glass house 19 days prior to inoculations.

Once germinated, all plants were fertilized fortnightly using Yates Thrive® (Nitrogen : Phosphorus ratio – 5:1).

2.2.2 Single-conidial isolates

The four isolates, NB053, NB085, SNB171 and SNB74S were retrieved from infected dry barley leaf pieces. Each leaf piece was immersed in 70% ethanol for five seconds, and then washed for two minutes in 5% sodium hypochlorite (NaOCl). Finally, the pieces were rinsed three times in distilled water. Each leaf piece was then placed on a filter paper saturated with distilled water in a petri dish and incubated for 12h photoperiod of normal light per 24 hour cycle at room temperature (Lehmensiek *et al.*, 2010). Leaves were daily monitored for appearance of conidia and once conidia were detected, a single conidium was picked using a sterile needle and plated on a Potato Dextrose Agar (PDA) plate. The plates were incubated at 22°C with 12hr alternating light and darkness for five days to obtain mycelial culture.

2.2.3 Inoculum preparation

Conidia were cultured by placing five plugs of each mycelial culture on the margins of sterilized barley or wheat leaf segments placed on 2% water agar medium (Fig. 2.1) for 10 days at 22°C prior to inoculation, under natural day light conditions (Deadman & Cooke, 1985; Ismail *et al.*, 2014). To remove the conidia, each plate was rinsed with ten mL of distilled water and conidia dislodged with a sterile paint brush. This solution was filtered through a fine sieve into a flask to be counted. The conidia were quantified using a haemocytometer (Weber and Sons, UK) and adjusted to a concentration of 5000 conidia/mL and three drops of 6% Tween 20 solution was added to each suspension. Spores were stored for a maximum of 2 months at –70°C before inoculation (Knight & Sutherland, 2013).

Fig. 2.1 Barley leaf-agar plates for culturing *Pyrenophora teres* conidia

2.2.4 Preparing the trays

2.2.4.1 DLA – droplet method

This method is based on the method described by Afanasenko *et al.* (2007). Fully expanded second leaves were cut into three cm segments and five segments of each variety were placed into 150mm diameter petri dishes on two sheets of filter paper moistened with 25 mL sterile water containing 0.004% benzimidazole. The leaf segments were arranged into groups with the adaxial side of the leaf uppermost as illustrated in Figs. 2.2 & 2.3. For the DLA - droplet test, five leaf segments were used within each experiment, and the experiment was repeated four times.

Tray 5	11	
	Keel	
Skiff	CI 5791	NRB11313
Westminster	Prior	Dash
	Skipper	

Fig. 2.2 DLA - droplet method template. The number on top (11) indicates the isolate's name. The tray's number in the 1st row indicates the position of the tray in the incubator.

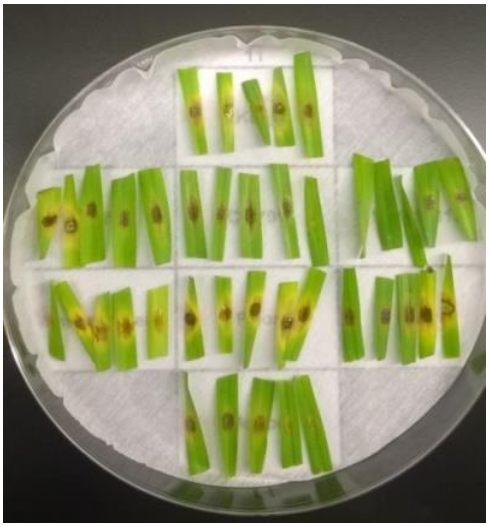


Fig. 2.3 DLA - droplet method inoculated. Five segments of 1.5-2 cm of the 2nd leaf of each variety are arranged into groups with the adaxial side of the leaf uppermost.

2.2.4.2 DLA – spray method

For the spray method, whole second leaves of the eight barley varieties were taped in groups of three onto square petri dishes (22.5 x 22.5 cm) with the top side of the leaf facing upward (Figs. 2.4, 2.5). Each tray contained filter paper moistened with 30 mL sterile water containing 0.004% benzimidazole. While three leaves were replicated within each DLA – spray test, the test itself was repeated four times.

NRB11313			CI5791			Westminster			Skiff			Skipper			Dash			Prior			Keel		
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
SNB74																							

Fig. 2.4 DLA - spray method template. The 1st row indicates the names of the varieties. The last row indicates the name of the isolate used for inoculation.

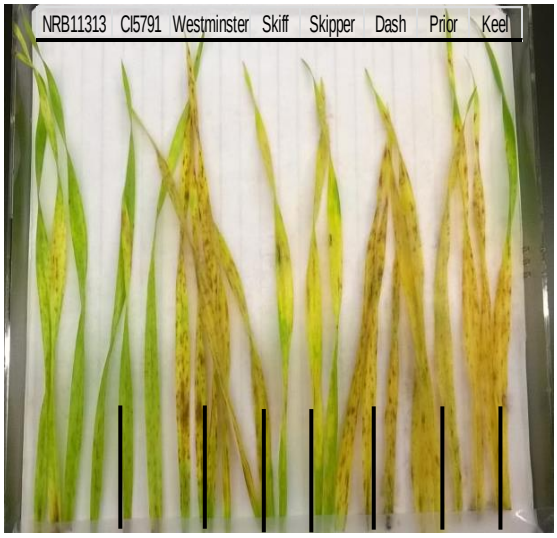


Fig. 2.5 DLA - spray method inoculated. Three 2nd leaves of each variety are arranged into groups with the adaxial side of the leaf uppermost.

2.2.5 Inoculations

To determine whether the conidia concentration used has significant effect on the disease severity score, plants were inoculated with different concentrations of isolate NB085; 2000, 5000, 10000, 15000 and 20000 conidia per mL. One isolate, NB085 was screened over four barley varieties: Dash, Keel, Skiff and Grimmette. Results suggested that disease severity scores were fairly consistent between the concentrations 5000 and 15000 conidia per mL. By parsimony, we used the lowest yet functional concentration; 5000 conidia per mL.

2.2.5.1 Seedling assay

Twenty mL of the 5000 conidia/mL conidial suspension prepared above was sprayed onto seedlings using Preval® spray gun. The pots were then placed randomly in a dew chamber for 24h at 100% RH and 23°C, and then moved

to a glasshouse at room temperature (20-24°C), for 14 days before scoring. Five seedlings of each variety were replicated within each test. The assay was repeated four times.

2.2.5.2 DLA – droplet method

A ten µL drop of the conidial solution was placed in the centre of each leaf segment (Fig. 2.6). Trays were placed into sealed plastic bags for maximum humidity and incubated at 22°C with 12hr alternating light and darkness for six days.

Fig. 2.6 Inoculation_ DLA - droplet method

2.2.5.3 DLA – spray method

For the spray method, ten mL of the same conidial solution used in the droplet method and the seedling assay were sprayed evenly onto the leaves using Preval® spray gun (Figs. 2.7, 2.8). Trays were placed into sealed plastic bags for maximum humidity and incubated at 22°C with 12hr alternating light and darkness for six days.

Fig. 2.7 DLA – spray method inoculated with *Pyrenophora teres f. maculata.* Advanced stage of the disease is shown with clear spot-like symptoms.

Fig. 2.8 DLA – spray method inoculated with *Pyrenophora teres f. teres*. Net-like, yellow and brown reticulations distinguishing NFNB symptoms can be clearly seen.

2.2.6 Disease rating

Disease scores were recorded 14 days after inoculation of the seedlings and six days after inoculation for the DLA assays. The Tekauz's (1985) seedling rating scale was used, where 1 is resistant and 9 and 10 is susceptible for

NFNB and SFNB, respectively (Appendix 2.1 and 2.2). For the DLA - droplet method the scale generated by Dr Anke Martin (unpublished data) was used (Appendix 2.3).

2.2.7 Data analysis

As the first aim of this study is to evaluate the possibility of replacing both, the seedling assay and the DLA – droplet method with the DLA – spray method for phenotyping net blotch of barley, Spearman's Rank correlation coefficient was used to statistically analyse the data obtained. This method is used to prove or disprove a hypothesis by identifying and testing the strength of relationship between two sets of data. In the case of this study, there are three sets of data to test the strength of relationship within each of them. These sets are: DLA – droplet method vs. DLA – spray method, DLA – droplet method vs. the seedling assay and DLA – spray method vs. the seedling assay. The Spearman's Rank correlation coefficient analysis was performed using Microsoft Excel®.

To evaluate each of the DLA assays in comparison to the seedling assay we performed contingency chi-square table using the seedling assay as the gold standard test to determine the specificity and sensitivity of each tested DLA methods. In this test, the sensitivity expresses "The ability of the test to identify correctly those who have the disease" (Susceptibility), while the specificity is "The ability of the test to identify correctly those who do not have the disease" (Resistance) (Kanchanaraksa, 2008; Kattan & Cowen, 2009; Shaikh, 2012). To apply this test it is required to have background information about the reactions of each genotype to each of the isolates screened. Thus, results from the seedling assay conducted at the USQ glasshouse facility were used for this purpose (Table 2.3). For the test, the provided information from the seedling assay is called the "Gold standard" (Kattan & Cowen, 2009). Using a 2 x 2 table (Shaikh, 2012), we compared the results of each of the DLA phenotypic assays we were evaluating to the gold standard test (seedling assay).

2.3 Results

Evaluating different conidial concentrations showed considerable differences amongst different concentrations. For a concentration as low as 2000 conidia per mL, symptoms were not as distinguishable when compared to the concentration of 5000 conidia per mL (Fig. 2.9). A slight increase of the symptoms between concentrations 5000 and 15000 conidia per mL was evident. However, for the highest concentration used (20000 conidia per mL), the symptoms were not as severe as expected. As such, throughout all the following inoculations for both of the DLA methods and the seedling assay, we used the concentration 5000 conidia per mL as it is the lowest concentration that could show measurable symptoms coherent with higher concentration.

Fig. 2.9 Conidial concentration test for isolate NB085. Conidial concentration used is 5000 conidia per mL. Varieties tested from left to right: Dash, Keel, Skiff and Grimmette.

The reactions of the barley differential set to each of the isolates tested were assessed through the seedling assay carried out in the laboratory and the glass house (Table 2.3). Each test was replicated four times with five plants within each.

The results of the seedling assay were slightly different to the data obtained from the DAFQ. As shown in Table 2.2, there are no data for either of the SFNB isolates we tested. Also, only genotypes Cl 5791, Prior, Skiff had data for the NFNB isolates NB053 and NB085. Genotype Keel was screened only with NB085. According to the seedling assay, Cl 5791 is resistant to NB053 but intermediate to the net form isolate NB085. The DAFQ results show also that Cl 5791 is resistant to both NB053 and NB085. Prior is susceptible to one of the NFNB isolates, NB085 as it is in the DAFQ data. However, to NB053, Prior was resistant as shown in DAFQ data but intermediate when screened by the seedling assay. Skiff is susceptible to NB085, intermediate to NB053 in the seedling assay while susceptible and resistant to NB053 and NB085, respectively in the DAFQ results. Similar to the DAFQ results, Keel is susceptible to NB085 and intermediate to NB053. Westminster is susceptible to NB085 and intermediate to NB053. Dash also is susceptible to NB085 but intermediate to NB053. NRB11313 is resistant NB053 but intermediate to NB085. Skipper is intermediate to NB053 and susceptible to NB085.

Table 2.3 Reactions of the barley lines to the tested *Pyrenophora teres* isolates according to the seedling test carried out in the laboratory of USQ. The actual scores are in brackets. Scores between 0-3.9 indicate resistance (R), 4-4.9 indicates intermediate reaction (I) and 5-10 indicate susceptibility (S).

Barley variety	Reaction to NB053	Reaction to NB085	Reaction to SNB171	Reaction to SNB74S
Cl 5791	2.4	4.3	2.4	3
Prior	4.2	9	4.2	4.2
Skiff	4.5	6.6	3	2.9
Keel	4.8	7.3	3.3	3.5
Westminster	4.5	6.8	4	4.5
Dash	4.5	7.9	5.8	5.8
NRB11313	2.9	3.9	2.6	3
Skipper	4.9	5.8	2.4	3.3

Key	
Resistant	
Intermediate	
Susceptible	

Comparison of the three phenotyping methods indicated that the DLA droplet-methods could not be used to determine the form of the disease, i.e. either net- or spot-type, whereas with the DLA-spray method the two types could clearly be distinguished (Figs. 2.10 and 2.11). Average disease scores for all isolates and genotypes are presented in Table 2.4.

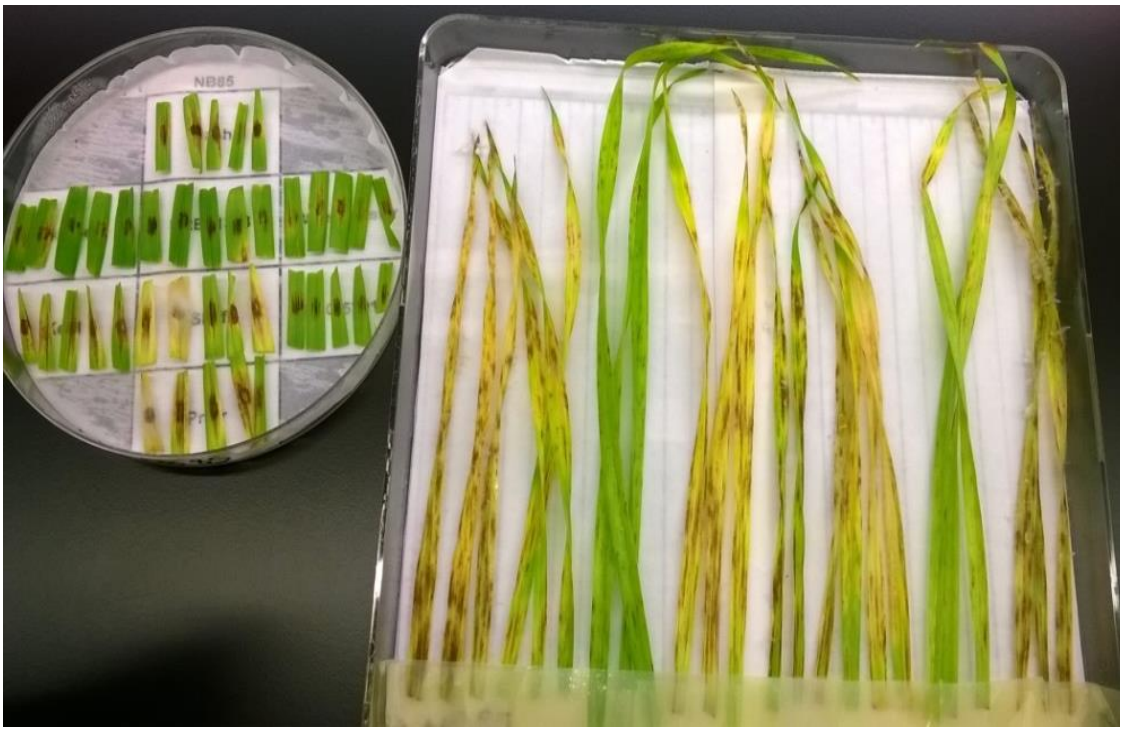


Fig. 2.10 DLA - droplet (to the left) and DLA - spray (to the right) inoculated with NFNB isolate, NB085. The order of varieties from left to right is: Skiff, Westminster, Prior, Keel, Skipper, NRB11313, Cl 5791 and Dash. Net-form symptoms are clearly distinguishable in the DLA spray method.

Fig. 2.11 DLA - droplet (to the left) and DLA - spray (to the right) inoculated with SFNB isolate, SNB171. The order of the varieties from left to right is: Keel, Skiff, Dash, Westminster, NRB11313, Cl 5791, Prior and Skipper. Spot-form symptoms are clearly distinguishable in the DLA spray method.

For both SFNB isolates SNB171 and SNB74S, the genotypes Cl 5791, Keel, NRB11313, Skiff and Skipper had scores ranging between 1.5 and 3.3. These scores were consistent across the three phenotyping methods. Inconsistent scores were observed with the genotypes Dash, Prior and Westminster between the different methods. For example, average scores between 2.7 - 2.9 were observed with genotype Dash for both SFNB isolates with the DLA – droplet method while scores between 5.2– 5.7 and 5.3 – 5.8 were observed with the DLA spray method and seedling assay, respectively. Similarly, genotype Westminster had scores between 1.9 – 2.7 with the DLA - droplet method while scores were 4 – 5 with the DLA-spray method and seedling assay.

Slight inconsistences were observed between the phenotyping methods when scoring the NFNB isolates. Genotypes Cl 5791 and NRB11313 scored 2.4 – 3 with isolate NB053 reflecting resistance. The same two genotypes Cl 5791 and NRB11313 showed different results upon screening with isolate NB085 with Cl 5791 scoring 2.8 with DLA-droplet method but 4.7 and 4.3 with the DLA - spray method and seedling assay, respectively. Similar results were observed with genotype NRB11313 and isolate NB085 with the three phenotyping methods with scores ranging between 3 and 4.3 for the different methods. In general, NFNB isolate NB053 displayed less virulence than isolate NB085 and was only observed to be moderately virulent (5.6) with Keel using the DLA - spray method.

Table 2.4 Average disease scores for all isolates and genotypes. Green highlighted cells represent resistant reaction, yellow highlighted cells represent intermediate reaction (moderate resistance - moderate susceptibility) and red highlighted cells represent susceptible reactions. Each score represents the average of 5 replicates in each trial for seedling assay and DLA – droplet test and three replicates for DLA – spray test.

Isolate	Genotype	DLA droplet average	DLA spray average	Seedling average
NB053	Cl 5791	2.7	2.4	2.4
NB053	Dash	3.0	4.7	4.5
NB053	Keel	3.0	5.6	4.8
NB053	NRB11313	2.6	3.0	2.9
NB053	Prior	2.8	3.8	4.2
NB053	Skiff	3.4	4.8	4.5
NB053	Skipper	2.8	4.7	4.9
NB053	Westminster	3.3	4.3	4.5
NB085	Cl 5791	2.8	4.7	4.3
NB085	Dash	4.5	9.0	7.9
NB085	Keel	5.7	8.5	7.3
NB085	NRB11313	3.0	4.3	3.9
NB085	Prior	6.2	9.3	9.0
NB085	Skiff	4.6	6.7	6.6
NB085	Skipper	3.1	7.3	5.8
NB085	Westminster	4.4	7.7	6.8
SNB171	Cl 5791	2.7	2.8	2.4
SNB171	Dash	2.9	5.7	5.3
SNB171	Keel	2.8	2.8	3.3
SNB171	NRB11313	2.5	2.4	2.6
SNB171	Prior	3.0	4.7	4.2
SNB171	Skiff	2.2	2.7	3.0
SNB171	Skipper	2.5	2.5	2.4
SNB171	Westminster	2.7	5.4	4.0
SNB74S	Cl 5791	2.1	2.8	3.1
SNB74S	Dash	2.7	5.2	5.8
SNB74S	Keel	2.3	2.7	3.5
SNB74S	NRB11313	2.2	2.5	2.9
SNB74S	Prior	2.6	3.5	4.2
SNB74S	Skiff	1.5	2.1	2.9
SNB74S	Skipper	2.5	2.3	3.3
SNB74S	Westminster	2.0	4.3	4.5

Similarly to the SFNB results discussed above, lower scores were observed with the DLA - droplet method than the two spray method and seedling assay for the genotypes Dash, Skiff, Skipper and Westminster when screened with isolate NB053. Amongst the four screened isolates, isolate NB085 was the most virulent. Differences were again observed between the DLA-droplet method and the other two methods. The scores for the two genotypes, Prior and Keel through all the assays was between 5.7 (Keel – droplet), 8.5 (Keel – spray) and 6.2 (Prior – droplet) and 9.3 (Prior – spray) showing consistent categorical assessment. Both genotypes were R but with a considerable gap between the 5 – 6 (moderate susceptibility) of the droplet and the 9 score (very susceptible) appearing in the spray method. The rest of genotypes; Dash, Skiff, Skipper and Westminster all showed moderate to high susceptibility with spray method and seedling assay with a range of scores between 5.8 and 9. However, the same genotypes showed different reactions with the DLA - droplet method. While Skipper was the only genotype showing low score of 3.1, the rest were showing scores of 4.4 – 4.6.

Looking at the correlation between results of each of the three phenotyping methods for each of the diseases, NFNB and SFNB showed higher correlation between the DLA – spray and seedling method than that between the seedling assay and DLA – droplet. For SFNB, the highest correlation was observed between the DLA – spray and seedling method Spearman's rho = 0.78, $\rho < 0.0004$ (Fig. 2.12 A). The correlation between DLA – droplet and DLA – spray was less, Spearman's rho = 0.61, $\rho = 0.013$ (Fig. 2.12 B), while a low correlation between DLA – droplet and seedling method was observed, Spearman's rho = 0.4, $\rho = 0.1194$ (Fig. 2.12 C).

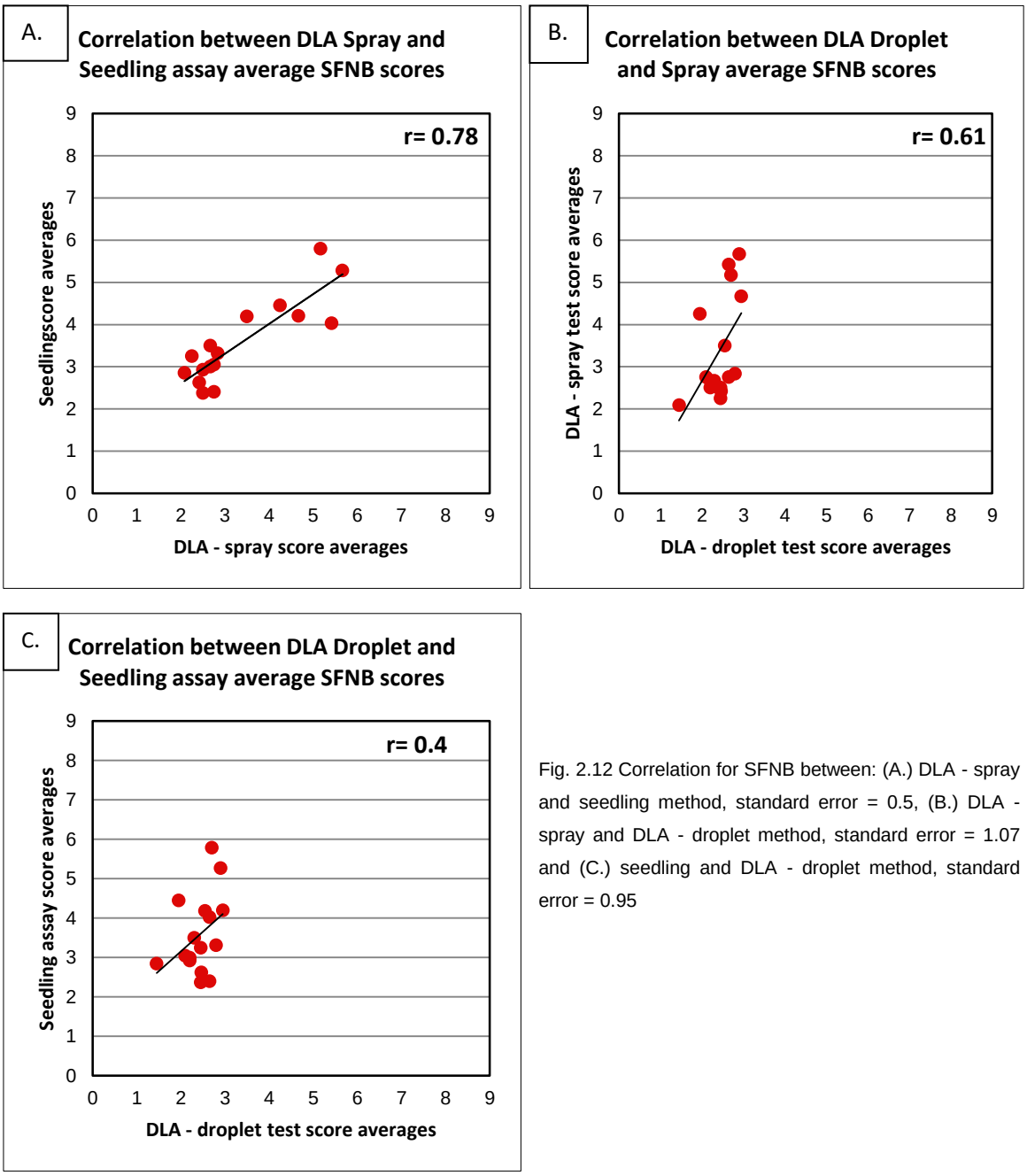


Fig. 2.12 Correlation for SFNB between: (A.) DLA - spray and seedling method, standard error = 0.5, (B.) DLA - spray and DLA - droplet method, standard error = 1.07 and (C.) seedling and DLA - droplet method, standard error = 0.95

For NFNB on the other hand, all the three methods were strongly correlated but also with the highest correlation between the DLA – spray and seedling method Spearman's rho = 0.97, $\rho < 0.0000000001$ (Fig. 2.13 A). The correlation between DLA – droplet and seedling method was Spearman's rho = 0.81, $\rho < 0.000001$ (Fig. 2.13 B), while it was less between DLA – droplet and DLA – spray, Spearman's rho = 0.83, $\rho < 0.00002$ (Fig. 2.13 C).

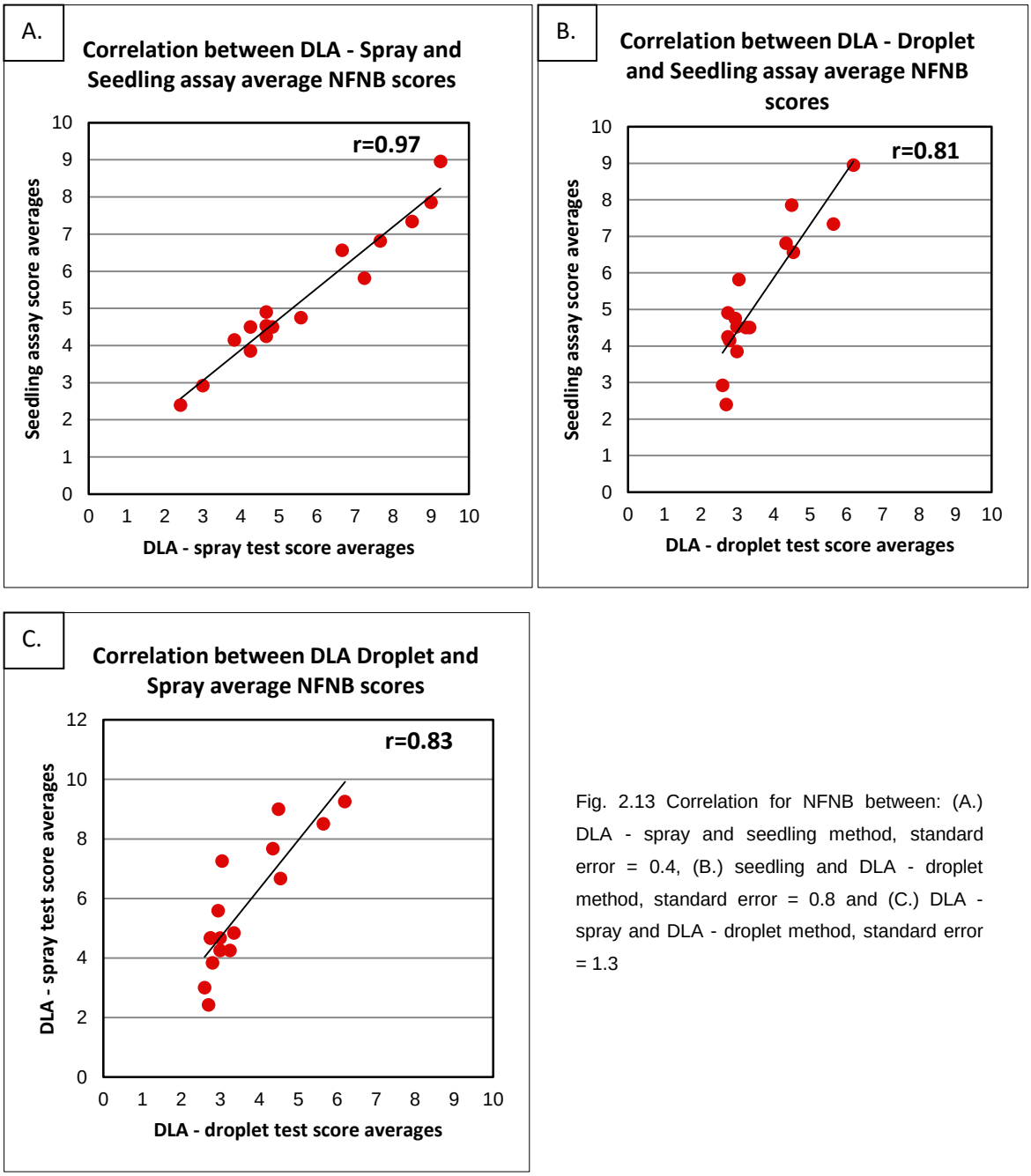


Fig. 2.13 Correlation for NFNB between: (A.) DLA - spray and seedling method, standard error = 0.4, (B.) seedling and DLA - droplet method, standard error = 0.8 and (C.) DLA - spray and DLA - droplet method, standard error = 1.3

As to the gold standard test, the results of DLA spray and droplet methods for NFNB were compared to the result of seedling assay for the same disease as the seedling assay was used as a standard. Due to the nature of the test, the results were divided either into positive/susceptible, above score of 5 or negative/resistant, below score of 5. Since only two lines were susceptible to the SFNB isolates the test was only applied to the NFNB. The test should reveal the sensitivity and specificity of each of the DLA method. As stated earlier, sensitivity expresses “The ability of the test to identify correctly those

who have the disease" (Susceptibility) (Kanchanaraksa, 2008). This is calculated by summing the number of times when the tested method, DLA droplet or DLA spray, was able to express susceptibility in a reaction. This number is compared to the number of susceptibility reactions in the standard method, the seedling assay. The more the sensitive method is the one that reflects as many of the susceptible cases as possible. The opposite of this is the specificity which is "The ability of the test to identify correctly those who do not have the disease" (Resistance) (Kanchanaraksa, 2008). In this case, the more specific method is the one that show as many resistant reactions as there are in the standard method.

Out of the 16 overall averages, seedling assay expressed the susceptibility of six samples and resistance of the other ten. The DLA - droplet (Table 2.5) has only expressed the susceptibility of two screened isolates/genotypes out of the six, while the DLA – spray method (Table 2.6) expressed seven susceptibilities. As to the resistant reactions, DLA droplet showed 14 resistant reactions out of the 16, while the DLA spray showed nine resistant reactions.

By calculating the sensitivity (ability to identify susceptible varieties), and specificity (ability of detecting resistant varieties), the DLA – droplet method had 33.3% sensitivity and 100% specificity. The DLA spray test however showed complete sensitivity, 100% and a high specificity, 90%.

Table 2.5 Contingency chi-square table representing the gold standard test to evaluate specificity and sensitivity of DLA droplet compared to the seedling assay for NFNB.

		SEEDLING ASSAY RESULTS	
		Susceptible (+ve)	Resistant (-ve)
Droplet test NFNB	Test results susceptible (+ve)	**True positive** (TP) = 2	**False positive** (FP) = 0
	Test results resistant (-ve)	**False negative** (FN) = 4	**True negative** (TN) = 10
		Sensitivity = TP / (TP + FN) = 2 / (2 + 4) **= 33.3%**	Specificity = TN / (FP + TN) = 10 / (0 + 10) = **100%**

Table 2.6 Contingency chi-square table representing the gold standard test to evaluate specificity and sensitivity of DLA spray compared to the seedling assay for NFNB.

		SEEDLING ASSAY RESULTS	
		Susceptible (+ve)	Resistant (-ve)
Spray test NFNB	Test results susceptible (+ve)	**True positive** (TP) = 6	**False positive** (FP) = 1
	Test results resistant (-ve)	**False negative** (FN) = 0	**True negative** (TN) = 9
		Sensitivity = TP / (TP + FN) = 6 / (6 + 0) **= 100%**	Specificity = TN / (FP + TN) = 9 / (1 + 9) **= 90%**

2.4 Discussion

Upon reviewing the literature, different researchers used different conidial concentrations for inoculations. This represented an issue to our study since we faced a large decrease in conidial yields during inoculum preparation during the preliminary tests. Consequently, we had to evaluate a range of concentrations to estimate the lowest, yet functional concentration possible to use to overcome the decrease in the conidial growth without affecting the symptom expression.

Testing different conidial concentrations has shown a considerable difference in symptoms between low concentrations, 2000 and 5000 conidia per mL with the latter only displaying distinguishable symptoms. The higher concentrations were easier to assess, however, at a concentration of 20000 conidia per mL, symptom expression decreased, probably due the excessive number of conidia competing to infect a relatively small area of the host. Since this test was carried out once, the ultimate concentration for use in inoculations requires further thorough investigation considering the different concentrations stated in literature, 4000 conidia per mL (Weiland *et al.*, 1999), 300000 conidia per mL (Jordan *et al.*, 1985), 1300 conidia per mL (Louw *et al.*, 1994) and 10,000 conidia per mL (Afanasenko *et al.*, 2007).

Comparing the results of the seedling assay to the results for same genotype/isolate obtained from the DAFQ showed inconsistencies. This could be due to the successive subculturing of the isolates as referred to by McDonald (1967). In his study, McDonald (1967) concluded that the successive subculturing of *P. teres* can result in modification in its sporulation and even virulence rate. Another reason could be the minor differences between different persons applying the same method. However, if that was a main reason most of the results of the seedling assay performed at USQ would have differed from those performed in DAFQ, which is not the case here. To avoid any misinterpretation as such, the seedling assay was repeated four times at the USQ with five replicates achieved each time. No significant variation was observed through all the reps and so the results were confidently used as standard.

According to the data obtained from the DAFQ and Tekauz's scale (1985), scores between 0 and 3.9 should indicate resistance to moderate resistance while the scores between 4 and 4.9 indicate intermediate reaction, moderate resistance – moderate susceptibility and scores above 5 would indicate moderate to high susceptibility. However, this ranking would not accurately reflect the pathogenicity of the fungus and/or the susceptibility of the host. For instance the gap between score 3.8 and 4.1 is only 0.4 but yet it changes the rank of the reaction from resistant to intermediately susceptible. While the gap between 5.1 and 9 is almost 4 but the rank would be the same, susceptible. As to the phenotypic methods evaluation, the three methods showed strong correlation for the NFNB. However, poor correlation was observed between the DLA – droplet method and the other two methods for SFNB. This complies well with the fact that the highest correlation is the one between DLA spray and seedling assay for either of the diseases. A highly significant correlation was observed between the seedling assay and the DLA spray method, r = 0.97 and 0.87 for NFNB and SFNB, respectively. This indicates the high reliability of the DLA - spray method. The overall ratings in the DLA droplet method were generally lower than those for the spray method and the seedling assay, providing an unacceptable level of false negatives when attempting to identify susceptible host genotypes.

This weak correlation between the DLA – droplet method and the other two methods might be due to the nature of the technique itself. Unlike the DLA – spray and the seedling assay, the DLA – droplet method uses small segments of leaves that are inoculated with single drops of the conidial suspension. This concentrates the pathogen's activity within this spot, whereas in the other two methods, the inoculum is sprayed and distributed across the leaf's surface allowing higher distribution of the pathogen and consequently more fungal growth, toxin production and eventually a higher score for the disease. Also, while the same conidial concentration was used for each of the three methods, this concentration was located in one spot in case of the DLA – droplet method resulting in greater fungal growth and toxin production then necrosis in one spot as opposed to many spots in the DLA-spray method giving the leaf a greater chance to defend itself. Such accumulation gives a false reflection about the disease severity.

Other than the reliability of the DLA - spray method compared to the DLA – droplet method, the former also allows for the symptoms to be identified. Both the net reticulations and elliptical spots for NFNB and SFNB, respectively were clearly identified by the DLA – spray method in contrast to the DLA - droplet method(Figs. 2.10 and 2.11). Thus the DLA – spray method is useful to determine the virulence and lesion types of hybrids whilst keeping the isolates confined in a laboratory.

Many studies were based on using the detached leaf assay, DLA droplet for screening other diseases such as *Fusarium* head Blight (FHB). However, most of those studies suggested the necessity of further investigation regarding the effectiveness of using this method. Upon studying partial disease resistance in barley to the FHB disease, Kumar *et al.* (2011) concluded that the DLA droplet method can be used for pre-screening of a wide range of genotypes to have a basic differentiation of the resistance of these genotypes. However, the DLA - droplet fails to express symptoms of certain species such as *Fusarium culmorum* compared to *F. graminearum* indicating the tendency of this method to provide false negatives for susceptibility (Kumar *et al.*, 2011). The same conclusion was revealed earlier through another study on FHB. In that study, Browne and Cooke (2005) observed that some wheat cultivars showed moderate susceptibility during the seedling assay while showing moderate resistance with the DLA droplet method. Those studies support the results of this study as they suggest that the use of the DLA droplet method may not be an adequate substitute for standard whole plant phenotyping methods. Together with the observation that the DLA droplet method cannot differentiate between spot form and net form lesion types, our results suggest that the DLA spray method is a more functional and informative rapid test than the DLA droplet method.

The necessity for any DLA to distinguish between net form and spot form lesions was an essential criterial for use in the detection of hybrids between the two forms. Consequently the DLA spray method has been applied in the work described in the following Chapters.

3 Chapter 3: Assessing the virulence of progeny produced from sexual recombination between different isolates of *P. teres*

3.1 Introduction

Sexual reproduction within the two forms of *P. teres* plays an important role in shaping the genetic population structure of the pathogen. Sexual reproduction between the two forms, although rare, has also been reported (Campbell *et al.*, 1999 ; Campbell *et al.*, 2002; Crous *et al.*, 1995; McLean *et al.*, 2014). The ability of *P. teres* to reproduce sexually both within and between the two forms has the potential to result in new virulences that could reduce the effectiveness of existing resistant barley varieties. Therefore, the second aim of this study is to investigate the new virulences that may arise from the *in vitro* crossing between two *Ptt* isolates of known pathogenicity and also different isolates of *Ptt* with *Ptm* to provide a wider and better understanding of *P. teres* virulences.

HYPOTHESIS:

Hybrids of P. teres express virulences different to their parents'.

3.2 Materials and methods

3.2.1 *In vitro* mating

To test virulences of hybrids, progeny of three different *Ptt* x *Ptm* crosses and one *Ptt* x *Ptt* cross made by Dr Martin, Centre for Crop Health, USQ, were used. Each population was given a specific number as illustrated in Table 3.1. Details for the isolates used for the crosses are listed in table 3.2 (Lehmensiek *et al.*, 2010).

Table 3.1 *In vitro* crosses between *Ptt x Ptt and Ptm* x *Ptt*

Name of population	Parent #1	Parental Form	Parent #2	Parental Form	Number of progeny
9	NB029	*Ptt*	NB085	*Ptt*	83
26	SNB74S	*Ptm*	NB053	*Ptt*	21
27	SNB171	*Ptm*	NB053	*Ptt*	19
37	SNBHRS07033	*Ptm*	NB63	*Ptt*	40

Table 3.2 List of the *Pyrenophora teres* isolates used for making *in vitro* crossings, with leaf-symptoms observed, geographic origin, year of collection and host (Lehmensiek *et al.*, 2010).

Isolate	Symptoms	Sampling location/state	Town	Year
NB029	*Ptt*	WA	Wongan Hills	1985
NB053	*Ptt*	SA	Narracoorte	1994
NB063	*Ptt*	WA	15 km N of Williams	1994
NB085	*Ptt*	QLD	Gatton	1995
SNB171	*Ptm*	WA	Palinup River	1995
SNB74S	*Ptm*	QLD	Millmerran	1995
SNBHRS07033	*Ptm*	QLD	Comet	2007

The progeny of each cross were given a serial decimal number that follows the number of the cross; e.g. for the NB053 x SNB171 cross, population 27, the progeny were given numbers 27.01, 27.02 up to 27.19.

Plates for crossings were prepared by laying autoclaved barley straw pieces on Sach's agar (Hebert, 1971) in petri dishes. Plates were inoculated by placing 25mm^2 plugs of mycelium from each of two isolates on opposite sides of the barley straw. Petri dishes were sealed by PARAFILM® M (Merck Pty Ltd) and placed into plastic bags to prevent desiccation of the agar. Then they were incubated at 15°C for 12 hours of alternating dark/light photoperiod. Plates were checked each week for the formation of mature

pseudothecia. Once mature pseudothecia were observed, i.e. pseudothecia were forming a short cylindrical beak or neck, water agar plates were placed on top of the crossing plate with the agar facing the pseudothecia. Plates were sealed with PARAFILM® M (Merck Pty Ltd) and returned to the incubator. Plates were checked each day for ascospores. Ascospores which had been ejected onto the water agar were removed with a sealed glass needle and single ascospores were transferred onto half strength concentration Potato Dextrose Agar (PDA) plates. These were incubated at 22°C until enough fungal mycelium had been produced for DNA extraction. Glycerol cultures were made of all isolates by placing 25mm^2 blocks of mycelium into a 1.5 ml tubes filled with 400 µL of 15% glycerol solution. Tubes were labelled and stored at -70°C.

3.2.2 Plant Material

The same varieties used for the assay comparison studies (Chapter 2) were used to screen populations 26 and 27, SNB74S x NB053 and SNB171 x NB053, respectively. The same differential set was also used to screen population 37, SNBHRS07033 x NB063 with the exception that variety NRB11313 was substituted with Fleet due to the lack of seeds of NRB11313 (Table 3.3). For population 9, NB29 (Beecher virulent) x NB085 (Prior virulent), a set of 15 different barley genotypes was used (Table 3.4). The barley differential set used included 12 lines previously tested at the Hermitage station (DAFQ) by Ryan Fowler and Greg Platz along with another three resistant lines.

Table 3.3 Reactions of the barley varieties to each of the parents of the *Ptm* x *Ptt* crosses. The results for NB053, SNB171 and SNB74S are based on the seedling assay carried out at the USQ. The results of SNBHRS07033 and NB063 are based on the Hermitage station trials

#	Genotype	Reaction to NB053	Reaction to SNB171	Reaction to SNB74S	Reaction to SNBHRS07033	Reaction to NB063
1a 1b	NRB 11313 (P. 26 & 27) Fleet (P. 37)	R	R	R	R	R
2	Cl 5791	R	R	R	unknown	unknown
3	Keel	R	R	R	unknown	unknown
4	Skiff	I	R	R	unknown	unknown
5	Skipper	I	S	S	unknown	unknown
6	Dash	I	R	R	I	S
7	Prior	I	S	S	S	S
8	Westminster	I	I	I	S	R

Table 3.4 The barley differential set used for screening *Ptt* x *Ptt* cross (NB29 x NB085). The reactions of barley varieties to the isolates are provided by Ryan Fowler and Greg Platz, the Hermitage station (DAFQ).

#	Genotype	Reaction to NB085	Reaction to NB029
1	Cl 11458	R	R
2	Algerian	R	S
3	Fleet	R	R
4	Beecher	R	S
5	Yerong	I	S
6	Maritime	R	S
7	Harbin	I	R
8	Prior	S	R
9	Corvette	S	R
10	Kombar	R	S
11	Skiff	R	R
12	Gilbert	S	R
13	Buloke	Resistant Differential	
14	Rojo	Resistant Differential	
15	Vlamingh	Resistant Differential	

3.2.3 Phenotyping

For screening populations 26, 27 and 37, the DLA – spray method was used to screen the hybrids and parents across the barley varieties. As for population 9, all progeny were previously screened by Dr Martin, Centre for Crop Health, USQ, using the DLA – droplet method (data not shown). However in this study, 30 isolates that showed inconsistent results, plus the parents were re-tested using the seedling assay. Procedures for obtaining single-conidium cultures, inoculum preparation, inoculation and scoring were followed as in Chapter 2, Pages 31-33.

To increase the production of conidia for population 37, different media were tested, including Sach's medium, PDA and 50% strength PDA. The media were tested over different isolates including NB085 and SNB74S. A block of each isolate was placed on two replicates of each medium. All the plates were sealed in plastic bags and incubated at 22°C with a 12h light period for two weeks.

3.3 Results

The seedling assay showed complete resistance of NRB11313 only while Cl 5791 was resistant only to NB053 but susceptible to SNB74S and intermediate to SNB171. Keel on the other hand was susceptible to NB053 but resistant and intermediate to SNB171 and SNB74S, respectively. Also, genotypes Dash and Westminster were susceptible to all the isolates though they were expected to be resistant to intermediate. Skiff was susceptible to NB053 but resistant to SNB74S and SNB171 while it was expected to be intermediate to NB053 and resistant to the SFNB isolates. On the contrary, Prior's results were close to those obtained from the Hermitage station where it was susceptible to SNB171 and SNB74S, but resistant to NB053. Compared to the reactions expected for parents in Tables 3.3 and 3.4, some inconsistencies between the seedling assay (Fig. 3.1) achieved in the USQ trials and those carried out in the Hermitage station were observed. For instance, genotypes NRB11313, Cl 5791 and Keel were expected to be resistant to all the three isolates. Many hybrids showed intermediate lesions

where the lesion type was unidentifiable as some genotypes had lesions with the spot-form and other genotypes had net-form lesions.

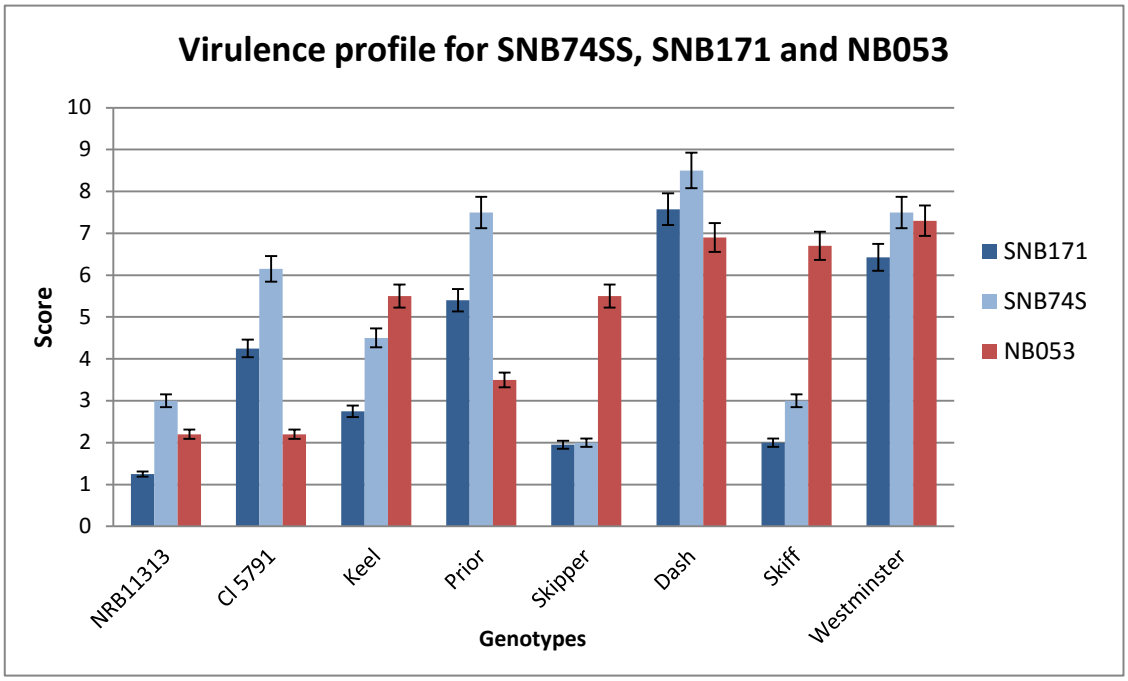


Fig. 3.1 Virulence profile for SNB171, SNB74S and NB053 - parents of populations 26 (SNB74S x NB053) and 27 (SNB171 x NB053) screened over eight barley varieties.

3.3.1 Population 26 (NB053 x SNB74S)

Scores for population 26 are listed in Table 3.5. The results show that none of the hybrids was virulent to genotype NRB11313. Genotype Cl 5791 showed similar resistance to NRB11313 except against the isolate SNB74S and four hybrids with spot-form type symptoms, 26.04, 26.05, 26.11 and 26.14. Westminster and Dash were the most susceptible genotypes to most of the hybrids. The most virulent hybrids according to the average score across all the genotypes were 26.04, 26.05, 26.09, 26.10, 26.11, 26.14 and 26.15 all of which showing spot-form lesions. However, hybrids 26.05, 26.10, 26.11, 26.14 and 26.15, all of which showed spot-form lesions were virulent on more than 50% of the genotypes. Hybrid 26.14 for instance was virulent to all the genotypes except NRB11313.

Table 3.5 Scores for population 26 (NB053 x SNB74S). The green coloured cells indicate resistant reactions; the red cells indicate the susceptible reactions while the yellow coloured cells indicate intermediate reactions.

Lesion type	Isolate	NRB11313	Cl 5791	Keel	Prior	Skipper	Dash	Skiff	Westminster	Isolate average
SF	26.13	1.00	1.50	3.00	2.00	2.15	3.50	4.00	3.15	2.54
Intermediate symptoms	26.02	2.10	2.50	2.35	2.00	2.50	3.50	3.00	3.00	2.62
SF	26.20	1.90	1.50	3.08	2.50	1.75	4.25	3.00	3.40	2.67
Intermediate symptoms	26.08	3.00	3.00	3.65	3.15	3.00	4.35	3.65	2.15	3.24
SF	26.21	1.00	2.65	2.15	3.00	3.00	3.35	6.00	5.15	3.29
SF	26.18	1.50	2.65	4.70	2.65	1.50	5.50	4.35	6.50	3.67
NF	26.16	1.00	2.00	6.00	2.85	2.00	4.50	4.65	7.15	3.77
SF	26.07	2.65	3.65	6.00	3.00	2.50	6.00	4.35	5.00	4.14
SF	26.03	2.00	4.15	4.00	5.00	2.00	7.00	3.30	5.85	4.16
SF	26.22	3.50	3.35	4.00	2.00	5.70	4.00	6.35	6.00	4.36
SF	26.19	2.58	3.75	4.65	5.50	2.50	5.60	4.50	5.90	4.37
SF	26.01	1.85	4.00	2.50	5.00	4.15	4.50	6.65	7.50	4.52
SF	26.06	3.00	3.50	4.00	6.50	2.30	7.30	4.35	7.00	4.74
NF	26.17	3.00	2.80	3.50	6.15	3.00	6.70	5.50	7.35	4.75
SF	26.23	3.50	4.35	4.35	3.00	5.15	5.85	6.50	5.85	4.82
SF	26.12	2.00	2.15	5.35	4.00	4.50	6.00	6.35	8.35	4.84
SF	26.15	1.80	4.15	5.85	5.50	2.65	6.50	5.00	8.00	4.93
SF	26.09	1.70	3.50	4.50	5.00	3.00	8.00	5.00	8.85	4.94
NF	NB053	2.20	2.20	5.50	3.50	5.50	6.90	6.70	7.30	4.98
SF	26.04	2.50	5.85	3.50	7.70	3.00	7.85	2.65	7.00	5.01
SF	26.05	1.50	5.00	4.50	6.35	4.00	7.85	5.00	6.00	5.03
SF	SNB74S	3.00	6.15	4.50	7.50	2.00	8.50	3.00	7.50	5.27
SF	26.14	2.00	5.00	5.00	5.35	5.35	7.15	5.35	9.00	5.53
SF	26.11	2.50	6.50	6.15	8.15	3.50	8.70	5.50	8.00	6.13
SF	26.10	2.50	4.00	5.50	8.50	5.50	8.00	6.50	9.00	6.19
	Genotype average	2.21	3.59	4.33	4.63	3.29	6.05	4.85	6.40	

Calculating the frequency distributions of infection scores for P.26 (Fig. 3.2), 60% of the reactions between hybrids and genotypes were resistant. Reactions of scores 5 and 6 had percentages of 15.2 and 14.8, respectively. Almost 10% of the reactions were very susceptible, ranging from 7 to 9.

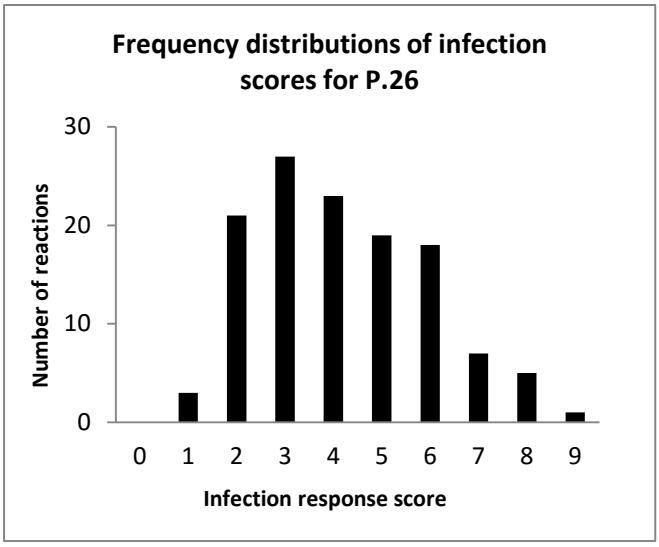


Fig. 3.2 Frequency distributions of infection scores for P.26. The distribution shows scores for all isolates on all genotypes.

Two hybrids, 26.02 and 26.08 showed intermediate lesion types which were intermediate between the spot form and net form lesions in appearance (Fig. 3.3). Both hybrids were avirulent to all the genotypes including the genotypes susceptible and/or intermediate to either of the parents (Fig. 3.4).

Fig. 3.3 Intermediate symptoms appearing in DLA – spray method, isolate 26.02. The order of the varieties from left to right is: Prior, Keel, Westminster and Dash.

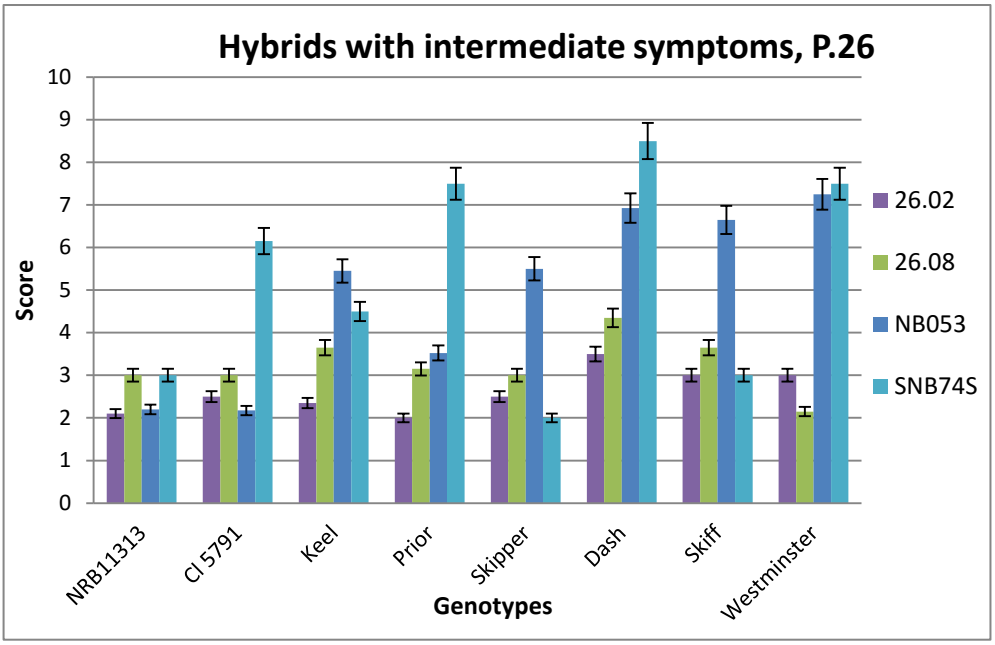


Fig. 3.4 Virulence profile of hybrids of P. 26 having intermediate symptoms.

No direct relationship was observed between the lesion type, (net, spot or intermediate) and the virulence. Though most of the spot-like hybrids resembled the virulence of SFNB parent SNB74S, two isolates, 26.13 and 26.20 were avirulent to all the screened genotypes in contrast to their virulence of both parents. Both isolates, 26.13 and 26.20, showed avirulence to Cl 5791 similar to the NFNB parent, NB053, but in contrast to the SFNB parent, SNB74S. However, both isolates were avirulent to Skipper, whereas only one of the parents, SNB74S was also avirulent to Skipper (Fig. 3. 5). Other genotypes showed mixed reactions where one of the isolates reflected the virulence of the NFNB parent while the other reflected the virulence of the SFNB parent. Hybrids with net-like symptoms also had different virulences to different genotypes compared to either of the parents (Fig. 3.6).

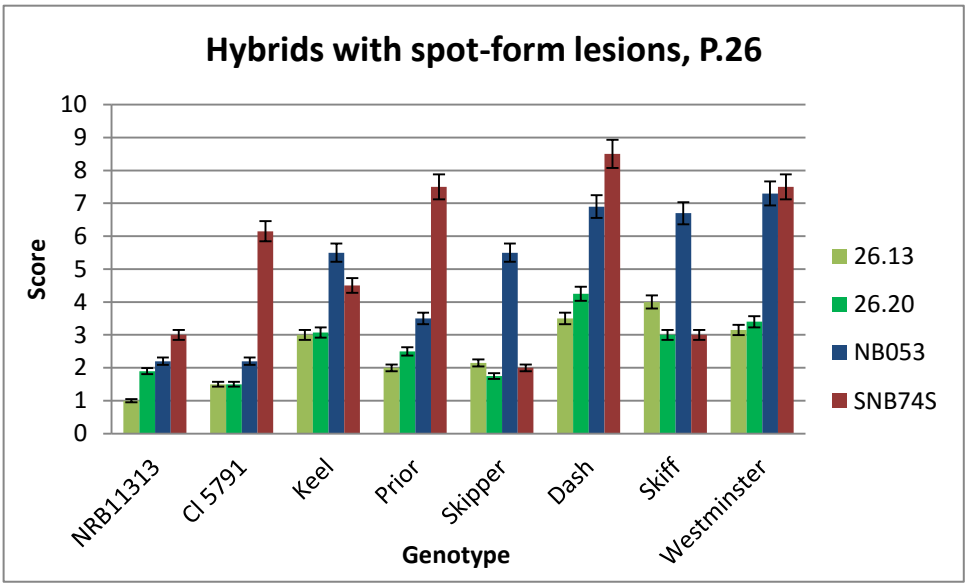


Fig. 3.5 Virulence of hybrids 26.13 and 26.20 and parents NB053 and SNB74S of P. 26 showing spot-form lesions.

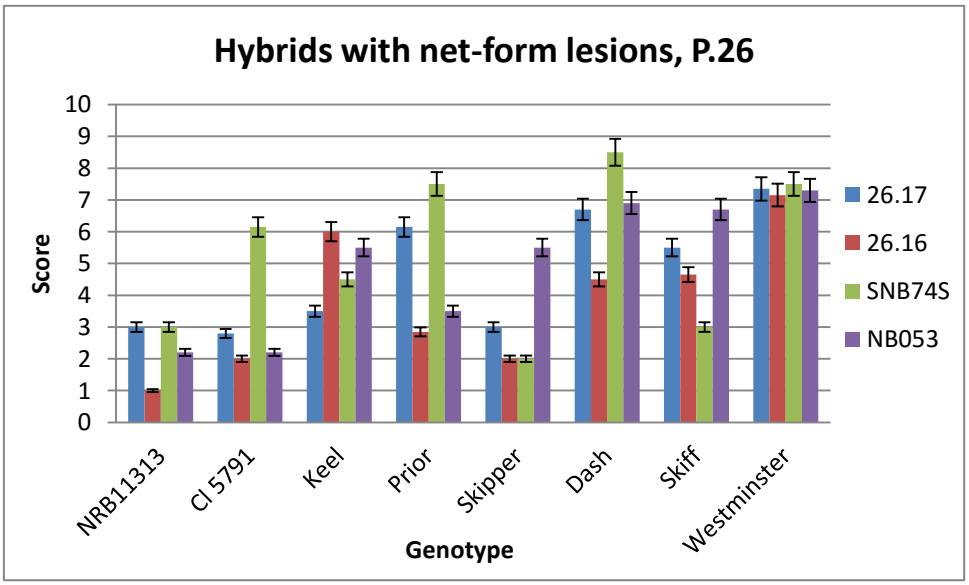


Fig. 3.6 Virulence of hybrids 26.17, 26.316 and parents SNB74S and NB053 of P.26 showing net-form lesions.

Hybrids with intermediate lesion symptoms, 26.02 and 26.08 were both avirulent on Prior, as was the NFNB parent, NB053 (Fig. 3.7).

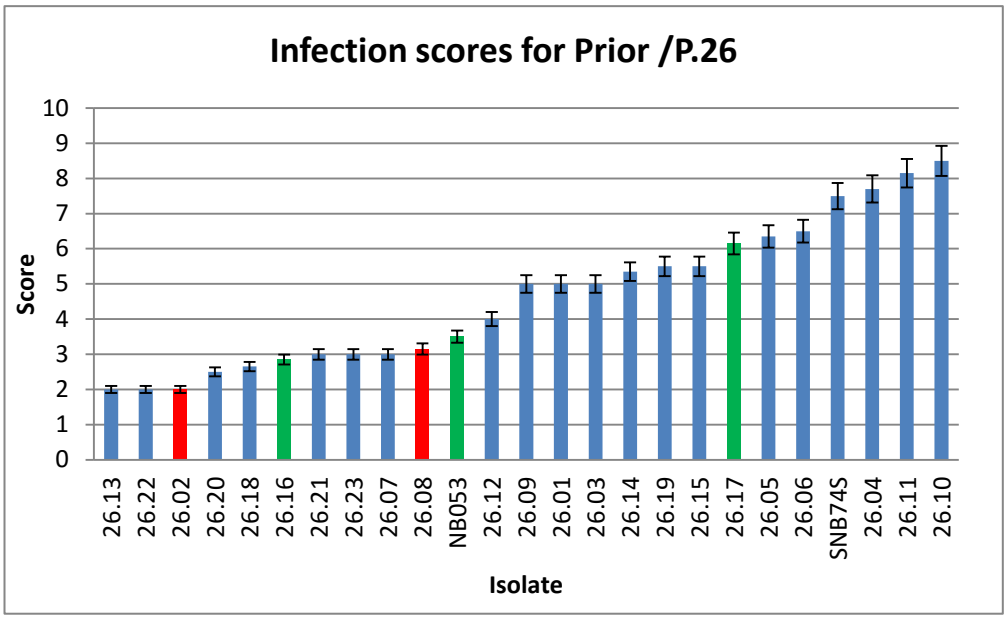


Fig. 3.7 Average scores for screening P.26 over genotype Prior. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

In contrast, intermediate hybrids, 26.02 and 26.08 were both avirulent on Dash and Westminster while both parents were virulent on these cultivars (Figs. 3.8 & 3.9).

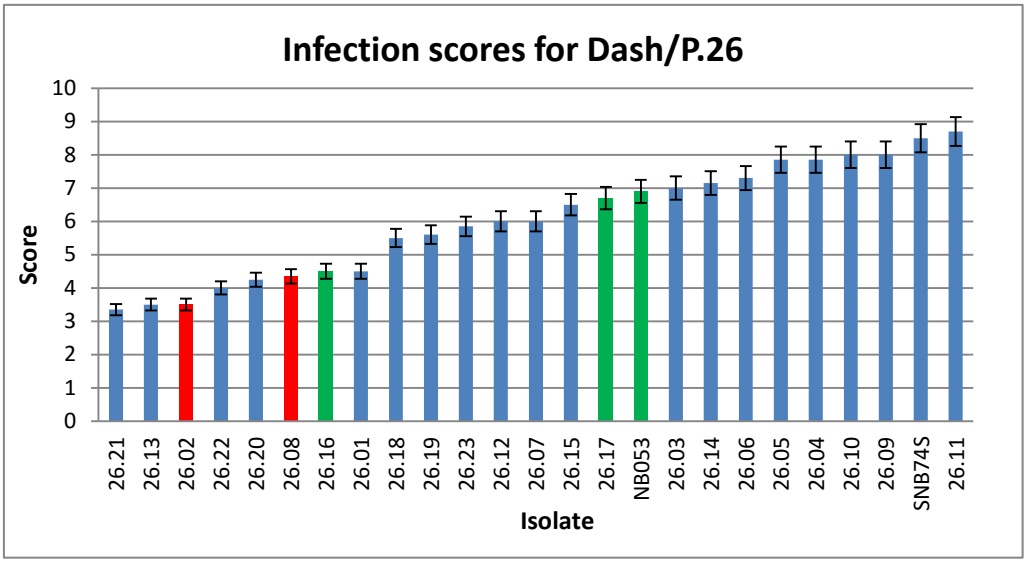


Fig. 3.8 Average scores for screening P.26 over genotype Dash. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

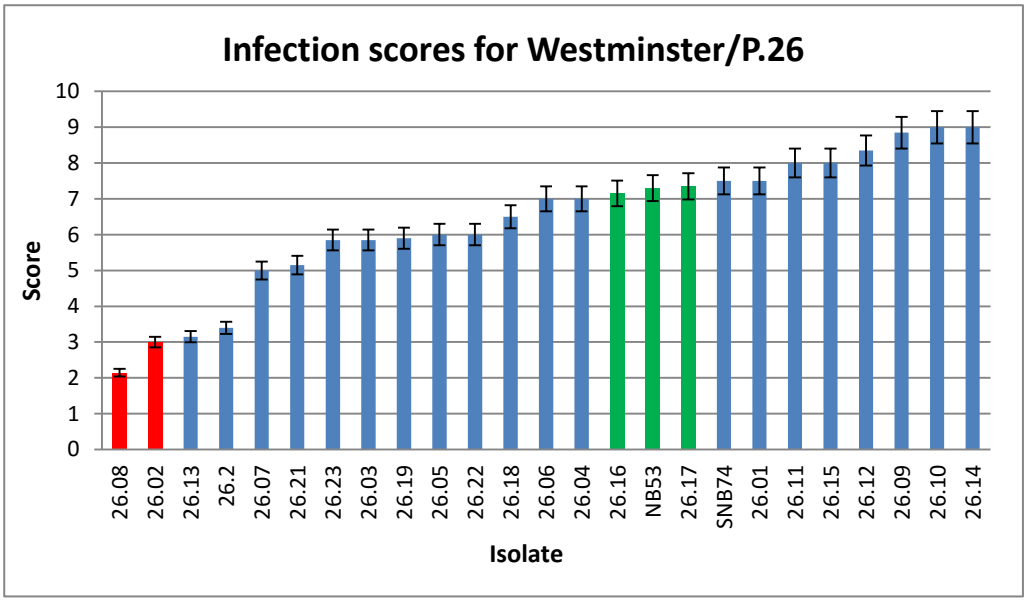


Fig 3.9 Average scores for screening P.26 over genotype Westminster. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

3.3.2 Population 27 (NB053 x SNB171)

The average scores for population 27 are listed in Table 3.6. None of the hybrids in this population were virulent on genotype NRB11313. Genotypes Skipper and Keel were also resistant to that population excluding the parent, NB053, which was virulent to both varieties. Dash and Westminster, however, are the most susceptible genotypes. Hybrids 27.13, 27.16 and 27.03, all of spot-form lesions, were the most avirulent hybrids as they barely caused infection to any of the genotypes. On contrast, hybrids 27.11 and 27.14, also of spot-form lesions, were the most virulent to genotypes CIho 5791, Westminster, Prior and Dash.

Table 3.6 Scores for population 27 (NB053 x SNB171). The green coloured cells indicate resistant reactions, the red cells indicate the susceptible reactions while the yellow coloured cells indicate intermediate reactions.

Lesion type	Isolate	NRB11313	Cl 5791	Keel	Prior	Skipper	Dash	Skiff	Westminster	Isolate average
SF	27.13	0.50	0.50	0.50	1.00	0.50	1.00	0.50	0.50	0.63
SF	27.16	1.00	2.00	1.00	2.00	1.50	2.15	2.00	3.00	1.83
SF	27.06	0.50	2.00	1.00	0.85	1.00	5.15	1.50	4.50	2.06
SF	27.09	1.00	2.50	1.65	2.50	1.00	3.00	1.50	4.00	2.14
Intermediate symptoms	27.08	1.00	2.65	1.50	1.50	1.50	5.65	2.00	1.85	2.21
SF	27.03	1.15	2.50	3.00	1.65	1.85	3.30	2.35	2.00	2.23
SF	27.18	1.50	2.85	1.00	3.15	0.75	5.00	1.00	3.75	2.38
SF	27.17	1.00	2.50	2.00	4.00	2.15	4.85	1.00	5.00	2.81
NF	27.07	0.50	2.15	4.00	1.80	1.65	4.00	5.65	4.00	2.97
SF	27.02	1.65	2.50	2.15	4.65	1.50	5.70	1.50	4.50	3.02
SF	27.04	1.65	3.35	1.75	3.85	0.75	6.15	1.50	5.50	3.06
SF	27.05	1.35	3.00	1.85	5.00	1.50	6.65	1.00	5.00	3.17
NF	27.10	1.00	3.65	1.85	4.00	1.00	8.00	1.00	6.65	3.39
SF	27.20	1.15	4.15	2.00	6.00	1.35	7.00	1.50	6.00	3.64
SF	SNB171	1.25	4.25	2.75	5.40	1.95	7.58	2.00	6.43	3.95
SF	27.21	2.00	4.35	3.35	4.50	3.35	5.35	2.75	6.10	3.97
SF	27.11	2.00	4.30	2.65	5.65	1.50	6.85	2.50	7.00	4.06
SF	27.14	1.85	5.15	2.35	7.35	2.15	6.00	2.00	5.70	4.07
NF	NB053	2.20	2.18	5.45	3.53	5.50	6.93	6.65	7.25	4.96
	Genotype average	1.28	2.98	2.20	3.60	1.71	5.28	2.10	4.67	

Almost 75% of the reactions between hybrids of population 27 and the tested genotypes were resistant (Fig. 3.10).

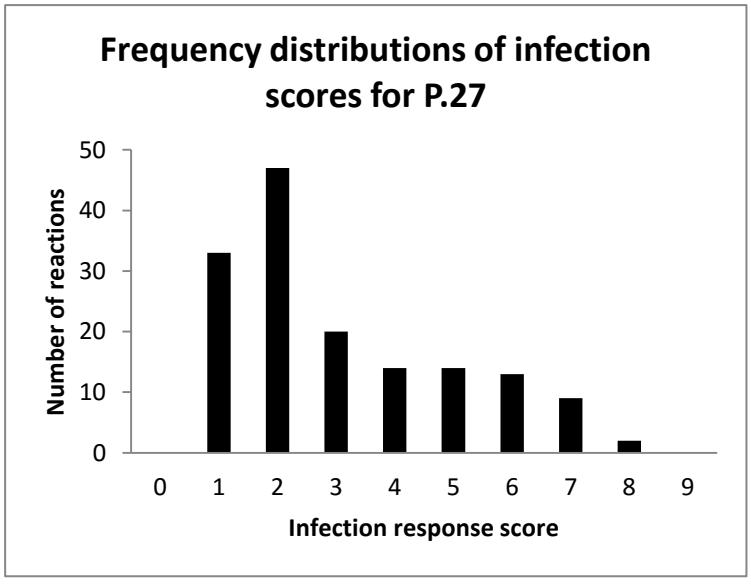


Fig. 3.10 Frequency distributions of infection scores for P.27. The distribution shows that most reactions between isolates and genotypes were highly resistant/avirulent. Eleven reactions only out of 152 were highly susceptible/virulent, while 26 were moderately susceptible/virulent.

One hybrid only, 27.08, showed intermediate symptoms (Fig. 3.11). That hybrid was avirulent on all the host genotypes except the cultivar Dash.

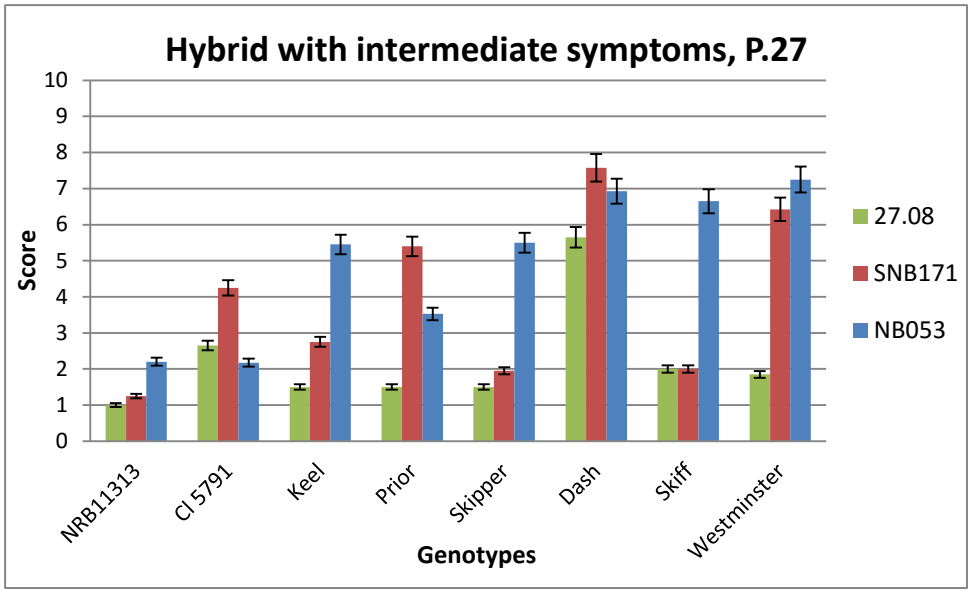


Fig. 3.11 Virulence of hybrid 27.08 and parents SNB171 and NB053 of P.27 showing intermediate symptoms

Two hybrids, 27.07 and 27.10 have expressed net-form type symptoms, but there was no relation between the lesion type and the virulence either (Fig. 3.12). Even though NB053 was virulent on all the genotypes except NRB11313, Cl 5791 and Prior, both hybrids were not as virulent. Hybrid 27.07 for instance was avirulent to all the genotypes except to Skiff. While 27.10 was avirulent only to two genotypes, Dash and Westminster. Most of the hybrids here showed virulence patterns different to that of either parent (Figs. 3.13, 3.14 and 3.15).

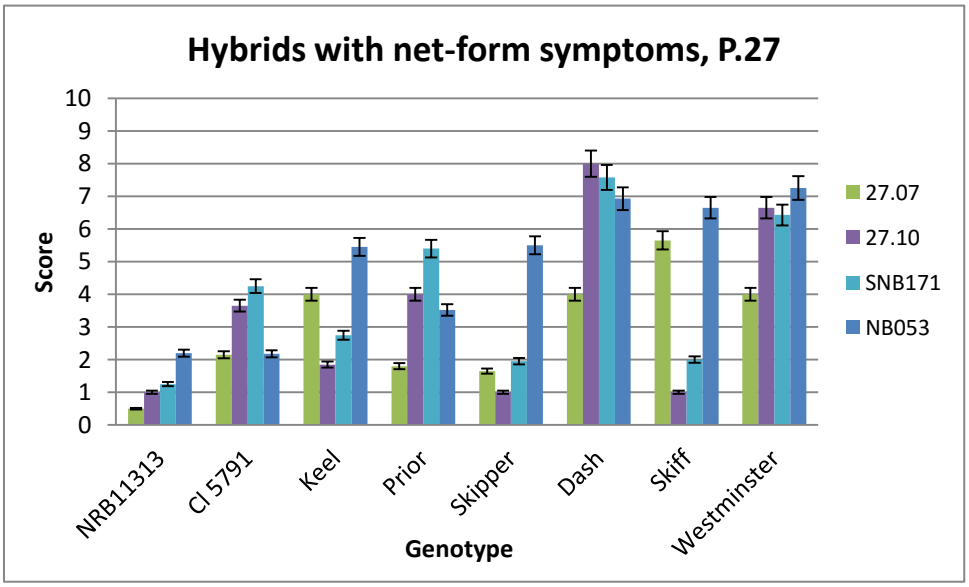


Fig. 3.12 Virulence of hybrid 27.08, 27.10 and parents SNB171 and NB053 of P.27 showing net-form lesions

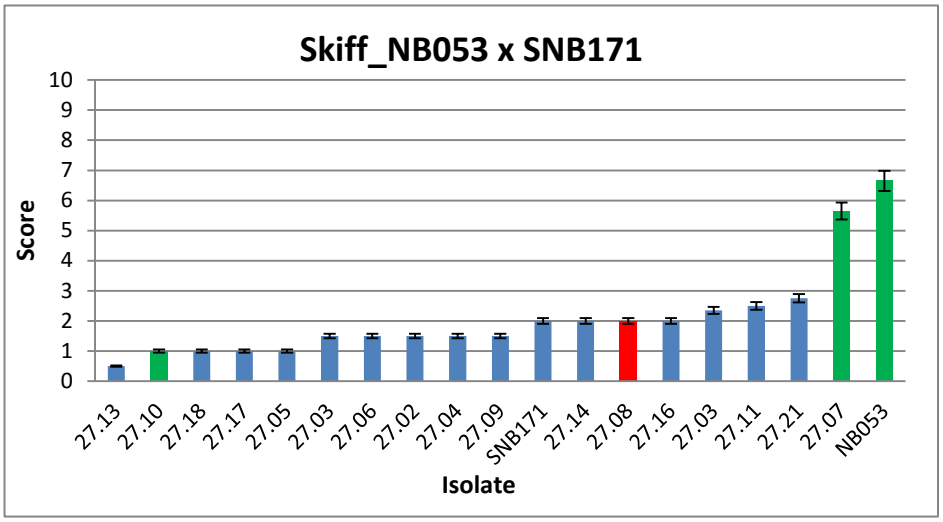


Fig. 3.13 Average scores for screening P.27 over genotype Skiff. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

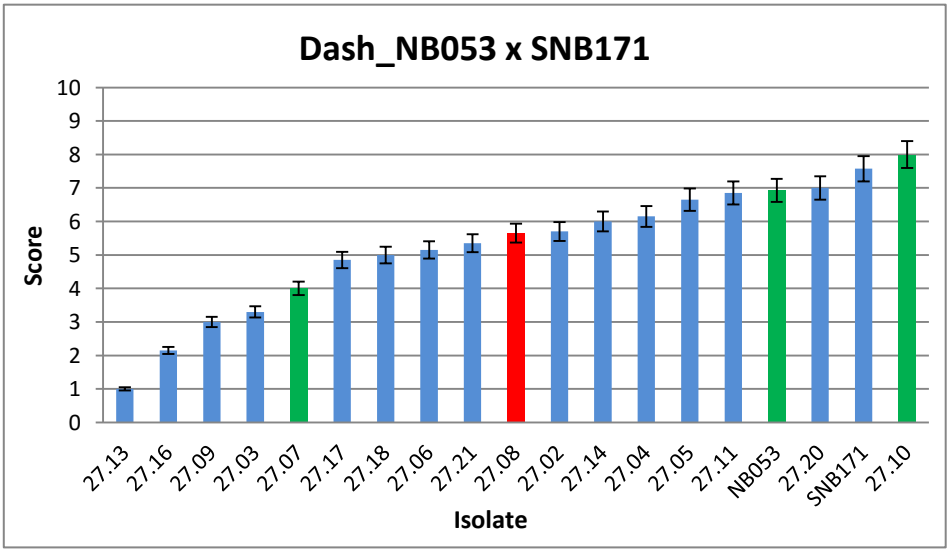


Fig. 3.14 Average scores for screening P.27 over genotype Dash. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

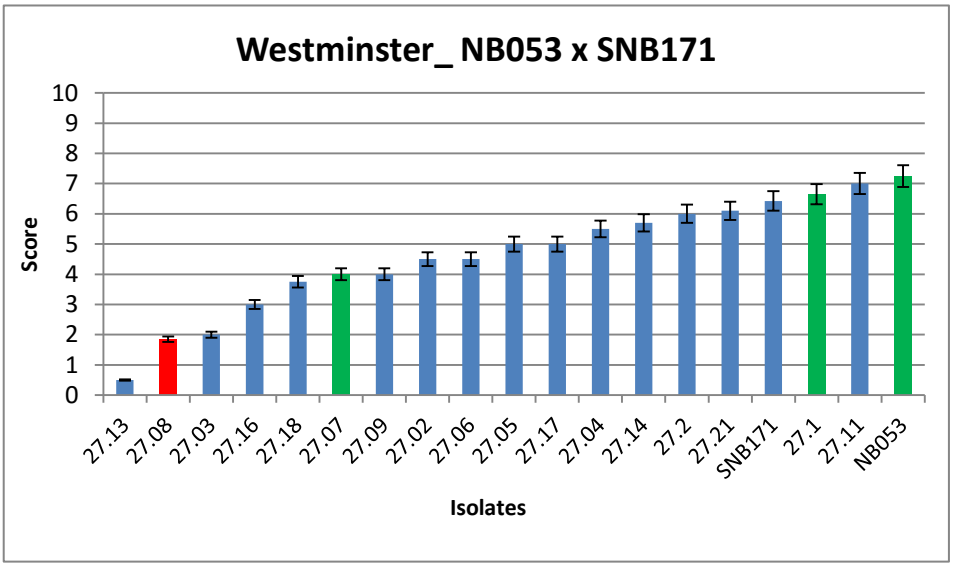


Fig. 3.15 Average scores for screening P.27 over genotype Westminster. Green columns represent lines with net form symptoms while red columns represent lines with intermediate symptoms. Blue columns represent spot form symptoms.

3.3.3 Population 37 (NB063 x SNBHRS07033)

Even though fresh mature barley leaves were used for conidial growth of population 37 (NB063 x SNBHRS07033), only three isolates (SNBHRS07033, 37.04 and 37.28) produced sufficient conidia for inoculation. Preliminary results of using different media for optimizing conidial growth suggest that the Sach's medium produced more conidia than the PDA. However, this observation needs to be further investigated.

Interestingly, both parents of population 37 were avirulent on all the genotypes (Fig. 3.16). The only exception is for the SFNB isolate SNBHRS07033 which was intermediate to the genotype CI 5791.

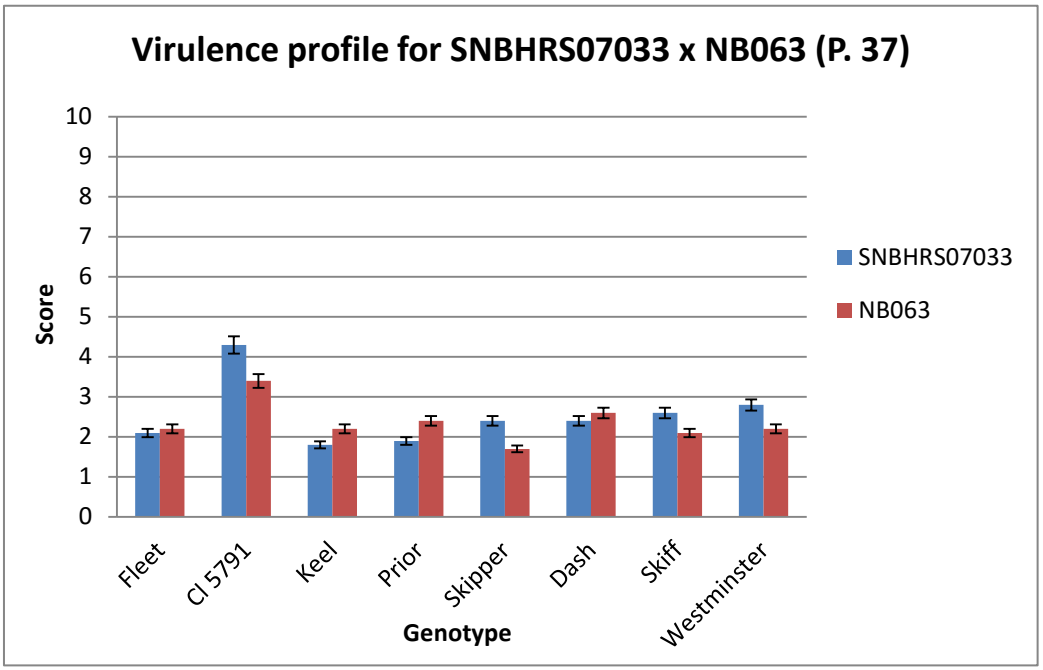


Fig. 3.16 Virulence profile for SNBHRS07033 and NB063 (P.37) screened over eight barley varieties.

All the isolates were totally avirulent except for one hybrid, 37.24 that was virulent on Prior, Westminster and Cl 5791. Genotype Cl 5791 was also susceptible to another three hybrids, 37.07, 37.30 and 37.13 (Table 3.7). Only 3.9% of the reactions showed virulence while the rest were highly avirulent/resistant (Fig. 3.17).

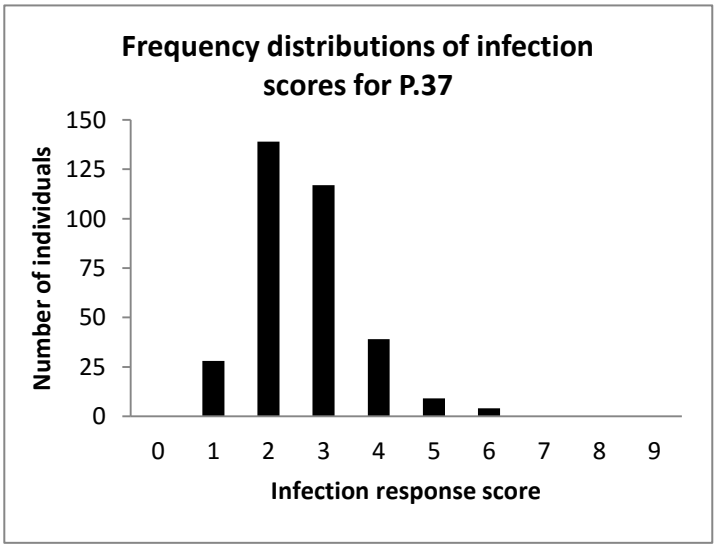


Fig. 3.17 Frequency distributions of infection scores for P.37. Only 13 reactions were moderately susceptible/virulent, while 284 reactions were highly resistant/avirulent.

Table 3.7 Scores for population 37 (NB063 x SNBHRS07033). The green coloured cells indicate resistant reactions; the red cells indicate susceptible reactions while the yellow coloured cells indicate intermediate

Lesion type	Isolate	Fleet	CI 5791	Keel	Prior	Skipper	Dash	Skiff	Westminster	Isolate average
SF	**37.21**	1.30	3.00	1.70	1.00	1.00	1.30	1.70	1.30	1.54
Intermediate symptoms	**37.22**	1.50	2.20	1.00	1.80	1.80	1.20	1.30	1.80	1.58
Intermediate symptoms	**37.08**	1.50	3.30	1.70	1.50	1.30	1.20	2.00	1.50	1.75
SF	**37.35**	1.80	3.30	1.20	1.30	1.50	1.20	1.80	2.00	1.76
SF	**37.27**	1.30	3.00	1.70	1.50	1.50	1.50	2.00	1.80	1.79
Intermedia te symptoms	**37.04**	1.50	2.70	1.20	2.20	1.30	1.50	2.30	2.20	1.86
NF	**37.23**	1.70	3.00	1.30	2.20	1.20	2.20	1.00	2.50	1.89
SF	**37.29**	1.30	3.70	1.50	2.50	1.30	1.80	2.20	1.50	1.98
NF	**37.20**	1.70	3.00	1.80	1.80	1.80	1.80	2.50	1.50	1.99
NF	**37.18**	1.70	3.70	1.30	1.80	2.00	1.50	2.50	1.70	2.03
NF	**37.17**	1.60	3.50	1.80	1.80	1.50	1.50	2.30	2.50	2.06
SF	**37.40**	2.00	2.80	3.00	1.70	1.80	1.70	1.80	2.50	2.16
Intermediate symptoms	**37.19**	2.00	3.70	1.50	2.00	1.70	1.80	2.50	2.50	2.21
SF	**37.07**	1.80	5.20	1.30	1.50	1.80	2.30	1.80	2.00	2.21
SF	**37.14**	1.50	4.20	1.00	2.00	2.00	2.50	2.20	2.50	2.24
NF	**NB063**	2.20	3.40	2.20	2.40	1.70	2.60	2.10	2.20	2.35
SF	**37.25**	1.30	4.00	2.00	2.00	1.80	3.20	2.80	1.80	2.36
SF	**37.09**	1.50	3.00	2.00	1.50	2.70	2.80	2.70	2.80	2.38
SF	**37.12**	1.80	3.80	3.20	1.30	1.50	3.00	2.30	2.70	2.45
SF	**SNBHRS0 7033**	2.10	4.30	1.80	1.90	2.40	2.40	2.60	2.80	2.54
SF	**37.38**	3.20	4.00	1.30	1.50	1.70	2.70	3.00	3.00	2.55
Intermediate symptoms	**37.03**	2.50	4.20	2.00	1.80	2.20	2.70	2.70	2.30	2.55
SF	**37.34**	1.80	4.80	3.20	1.30	2.20	2.50	2.80	2.00	2.58
SF	**37.10**	2.00	4.20	2.70	1.80	1.70	3.70	2.00	2.80	2.61
SF	**37.36**	2.30	3.70	2.00	2.20	2.80	2.70	3.50	2.00	2.65
Intermediate symptoms	**37.39**	2.00	4.30	2.20	1.80	2.70	2.80	2.70	2.70	2.65
SF	**37.28**	2.80	4.00	2.00	2.50	2.50	2.50	2.30	2.70	2.66
Intermediate symptoms	**37.05**	1.70	4.70	1.50	2.20	2.70	2.50	2.30	3.70	2.66
SF	**37.32**	1.80	4.50	2.50	1.70	2.20	2.80	2.70	3.30	2.69
NF	**37.30**	2.00	5.30	2.30	1.70	2.80	1.70	1.50	4.50	2.73
SF	**37.31**	2.50	3.50	2.00	1.70	2.70	3.70	3.20	2.80	2.76
SF	**37.16**	1.70	4.00	2.80	2.50	2.50	3.00	3.00	2.80	2.79
Intermediate symptoms	**37.06**	2.00	3.80	2.80	3.00	1.80	3.70	2.80	3.50	2.93
Intermediate symptoms	**37.15**	2.50	3.30	2.80	2.70	3.00	3.70	2.20	3.30	2.94
Intermediate symptoms	**37.33**	2.20	4.30	2.50	2.50	2.70	3.70	3.20	3.20	3.04

SF	**37.02**	2.20	4.80	2.50	2.80	2.50	3.30	3.00	3.30	3.05
NF	**37.26**	2.50	4.70	2.00	2.80	2.50	3.20	2.80	4.00	3.06
NF	**37.01**	2.70	4.00	2.20	3.20	3.00	2.50	3.30	3.70	3.08
Intermediate symptoms	**37.37**	2.70	4.20	2.70	2.80	2.70	3.50	3.30	3.50	3.18
SF	**37.11**	2.50	4.70	3.30	2.50	3.00	4.00	2.50	3.30	3.23
NF	**37.13**	3.20	5.50	3.00	2.30	3.80	3.50	2.20	4.30	3.48
NF	**37.24**	4.30	6.30	2.30	6.00	4.00	4.00	3.00	6.30	4.53
	Genotype average	2.05	3.94	2.07	2.12	2.17	2.56	2.44	2.74	

Around 13% of the progeny showed intermediate symptoms/lesions (Fig. 3.18) as the lesions were not distinguishable. None of the intermediate hybrids was virulent to any of the genotypes tested.

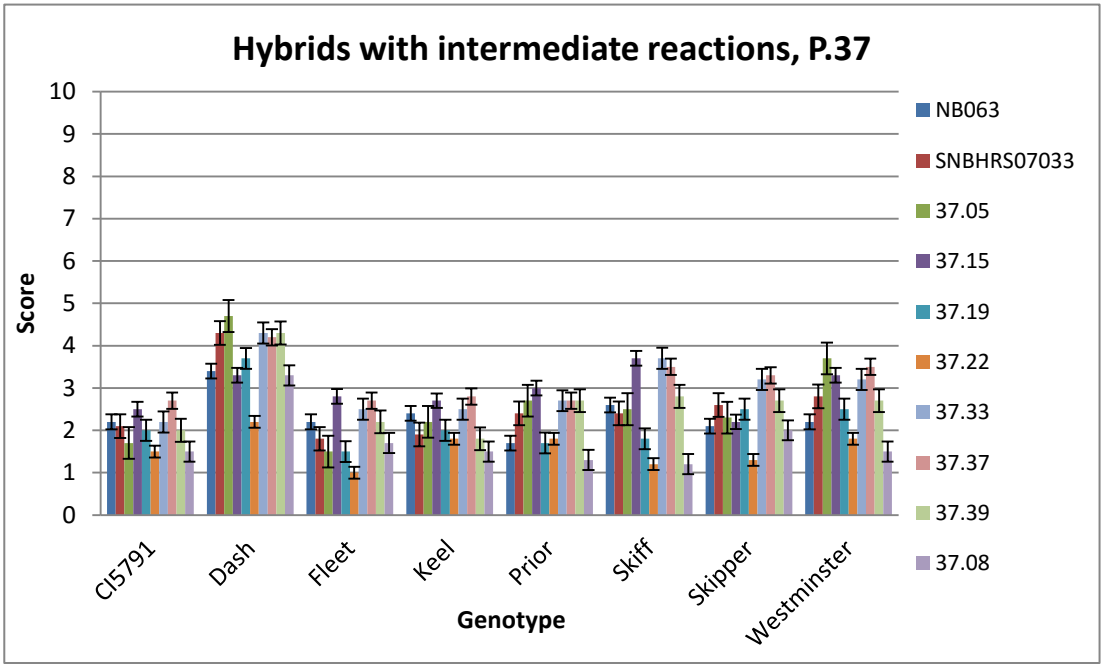


Fig. 3.18 Selected progeny of P. 37 showing intermediate reactions

3.3.4 Population 9 (NB029 x NB085)

For population 9 (Fig. 3.19), genotypes CI 11458, Kombar, Rojo and Vlamingh were resistant to both parents, NB085 and NB029, while only Corvette was susceptible to both parents and Fleet and Yerong intermediate. Skiff, Harbin and Buloke were resistant to NB029 while intermediate to NB085. Algerian and Gilbert were resistant to NB029 but susceptible to NB085. Prior was also susceptible to NB085 but intermediate toward NB029. Both genotypes Beecher and Maritime were susceptible to NB029 but with

Beecher being resistant to NB085 and Maritime intermediately reacting to NB085.

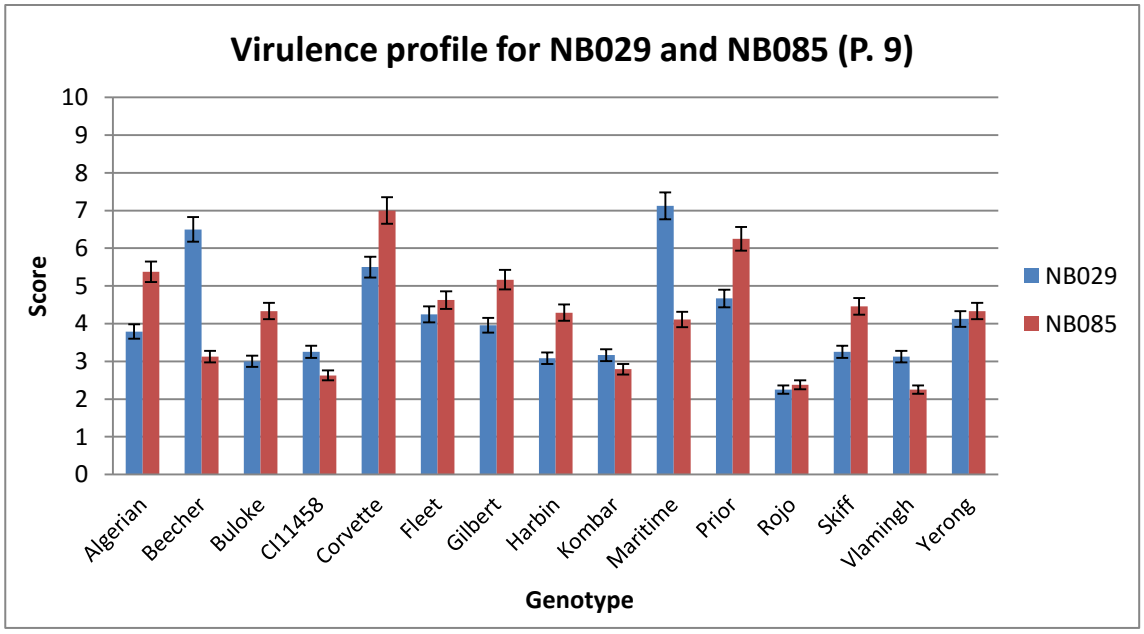


Fig. 3.19 Virulence profile for NB029 and NB085, parents of P.9 screened over 15 barley varieties.

The seedlings inoculated with hybrids of population 9 were assessed on the 14th day of inoculation (Appendix 3.1). Almost 70% of those seedling genotypes showed resistant reactions to the hybrids screened (Fig. 3.20). As to the genotypes averages (Table 3.9), Skiff and Gilbert were the most susceptible while Maritime, Buloke and Vlamingh were the most resistant.

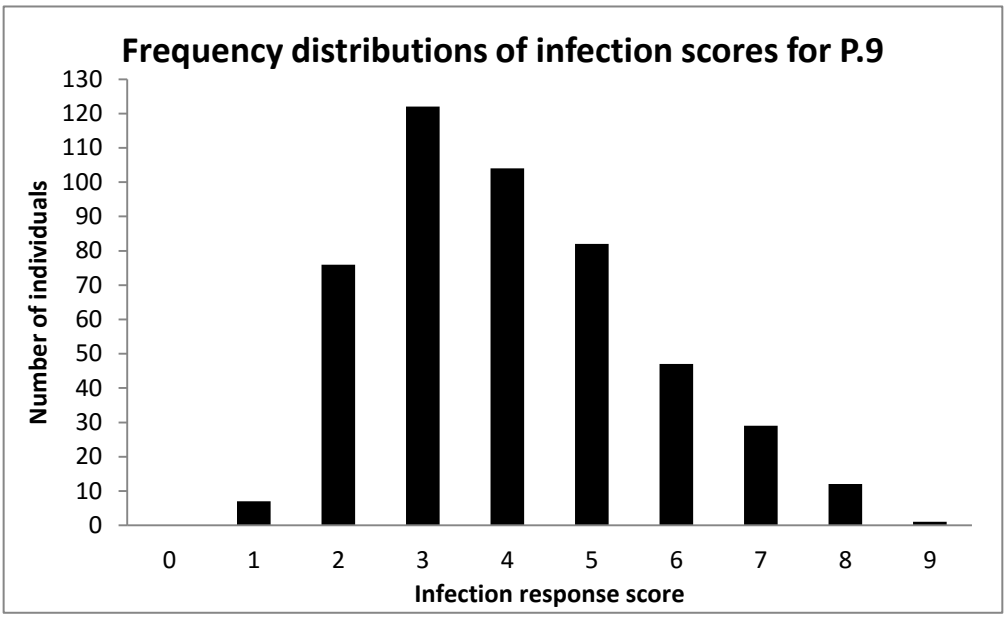


Fig. 3.20 Frequency distributions of infection scores for P.9. Out of 440 reactions, 131 were moderately to highly susceptible/virulent, while 205 reactions were highly resistant/avirulent.

Furthermore, the average scores of the screened isolates (Table 3.8) show that hybrids 9.16 and 9.21 are the most virulent, with an average of 5.2 for each exceeding the virulence range of both of parents. Hybrid 9.21 expressed virulence to resistant genotypes Kombar and Yerong in contrast to both parents which were avirulent to those genotypes. Also, hybrid 9.16 was highly virulent to genotype Yerong with a score ≈ 8, while both parents were avirulent to this genotype (Fig. 3.21).

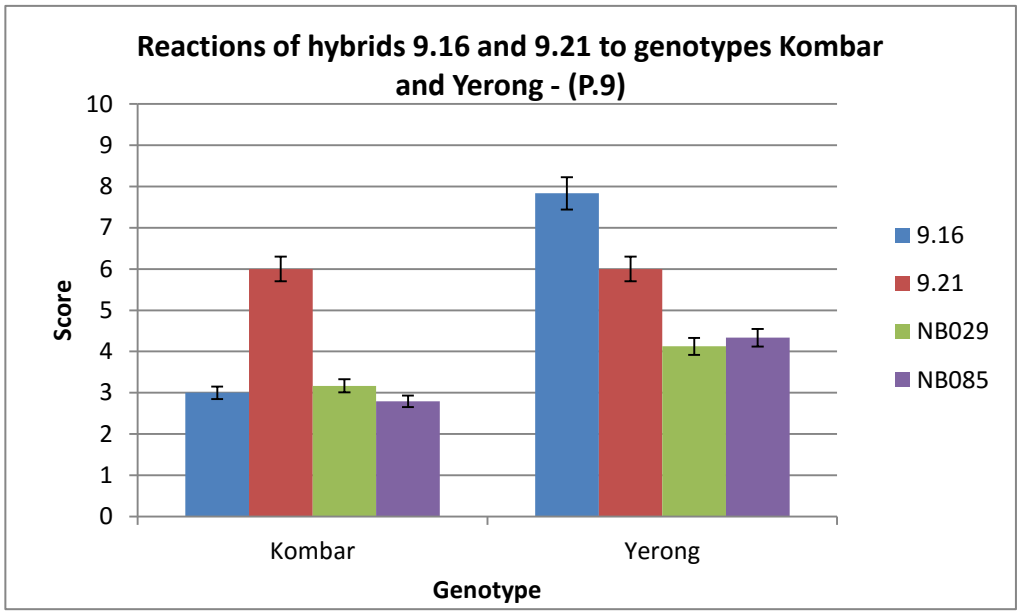


Fig. 3.21 Reactions of hybrids 9.16 an 9.21 to genotypes Kombar and Yerong compared to the reactions of both parents NB029 and NB085 (P.9).

Table 3.8 Average scores of isolates of population 9

Isolate	Isolate average
9.65	2.1
9.13	2.3
9.68	2.5
9.11	2.6
9.04	2.6
9.35	2.8
9.7	2.9
9.55	3.2
9.08	3.3
9.47	3.3
9.36	3.4
9.45	3.5
9.05	3.6
9.28	3.6
9.64	3.6
9.41	3.8
9.4	3.8
9.54	3.8
9.22	3.8
9.3	3.9
9.44	4
9.62	4
9.23	4.1
NB029	4.1
NB085	4.2
9.07	4.3
9.14	4.5
9.34	4.5
9.38	4.7
9.09	4.7
9.16	5.2
9.21	5.2

Table 3.9 Average scores of genotypes of population 9

Genotype	Genotype average
Maritime	2
Buloke	2.3
Vlamingh	2.4
Yerong	2.9
Corvette	3.1
Beecher	3.1
Fleet	3.3
Algerian	3.6
Prior	3.7
Kombar	3.9
Harbin	4
CI11458	4.3
Rojo	4.8
Skiff	5
Gilbert	5.3

3.4 Discussion

The second hypothesis that "*Hybrids of P. teres express virulences different to their parents'"* is verified; since some hybrids in each population examined have shown different reactions to those of their parents. Out of the 80 *Ptt* x *Ptm* hybrid isolates phenotyped, only 13 isolates (16.25%) showed net-like symptoms in infected host tissues. Another 13 hybrids had unclear lesion type and could not be identified either as net or spot type symptoms and were thus classified as intermediate. Eight of these intermediate hybrids were produced from population 37 only representing almost 10% of the overall count of hybrids. This high percentage might be due to the larger number of this population compared to populations 26 and 27.

The remaining hybrids had clear spot form symptoms. However, the hybrid lines in these populations showed virulence patterns on the host differentials that are mostly different to that of either parent. Since all hybrid lines showed only one type of lesion on all cultivars infected i.e. NF, SF, or intermediate, we would assume that this is controlled by fungal genes and not influenced by any host/fungal interaction, irrespective of the level of virulence expressed.

The overall behaviour of each hybrid, either in regards to its virulence or to its symptoms expression (lesion type) is mostly different from the parents. With the spot-like hybrids forming more than 82% of the progeny of the total *Ptt* x *Ptm* hybrids, the differences in virulence between the parents and the hybrids that resemble them in lesion type were emphasized among the spot-form hybrids. While SFNB parent SNB74S should be highly virulent on some varieties, certain hybrids derived from it, 26.02, 26.08, 26.13 and 26.20 were completely avirulent to all the varieties. The same case was repeated in population 27 where spot-like hybrids 27.03, 27.08, 27.13, 27.18 and 27.16 were avirulent to all genotypes unlike the SFNB parent SNB171.

In population 27, two isolates only showed resemblance to the net form parent in symptoms expression, while only one of those hybrids showed similar virulence/avirulence to that of parent NB053. Furthermore, this virulence/avirulence reaction differs according to the genotype tested; e.g.

while NB053 is virulent to Skiff and Westminster, the hybrids 27.10 and 27.07 showed virulence to only one of those genotypes: Westminster and Skiff, respectively. In the same population though, hybrid 27.08, which showed intermediate symptoms between net and spot lesions does not comply with either of the parents for all the genotypes. Even though this hybrid showed avirulence to Skiff like the spot form parent (SNB171), it showed avirulence to Prior like the net form parent (NB053). Moreover, both parents were virulent to Westminster and Dash, while hybrid 27.08 was only virulent to Dash but not to Westminster.

As to population 9, hybrid 9.21 expressed virulence to resistant genotypes such as Kombar and Yerong even though neither of the parents was virulent to those genotypes. Also, hybrid 9.16 was highly virulent to genotype Yerong unlike the parents. This illustrates the potential threat of having hybrid occurrence in the field – which despite its rarity, has already been reported more than once (Campbell *et al.*, 2002; Lehmensiek *et al.*, 2010; Leisova *et al.*, 2005; McLean *et al.*, 2014).

Despite the virulence of many of the isolates tested, the genotype NRB11313 was resistant to all isolates from populations 26 and 27 verifying its record as a resistant variety. Another variety is Cl 5791 which showed a significant resistance to more than 95% of the spot-form hybrids. Those two genotypes represent a rich resource for identifying QTL regions associated with resistance to both forms of *P. teres* leading to isolating and cloning those loci to develop resistant barley varieties.

During this study, a large decrease in the conidial production of the hybrids was detected. It was also observed that there are differences between results of the parental isolates screened in different experiments. For example, when screened on genotypes Cl 5791 and Prior, SNFB isolate, SNB74S showed an average virulence of 6 and 7.5 (highly susceptible), respectively, over three repetitions (Table 3.5). However, when the experiment was repeated later on the same year, the virulence score for Cl 5791 dropped to 3 (resistant) and 4.2 (intermediate) for Prior (Table 2.3). This probably was caused by a drop in the number of the conidia used in the conidial

suspension. The reduction in conidia cultured may be due to successive subculturing of *P. teres* which can affect the behaviour of the fungus; this behaviour includes the sporulation rate as well as the virulence of the pathogen (McDonald, 1967). As such we carried out some experiments using subcultured inoculates and re-grown inoculates from frozen cultures. Unfortunately, no significant difference was detected.

Conidia production methods vary between different groups. Weiland *et al.* (1999) cultured *P. teres* on V8 juice agar and incubate it at 22°C with a 12h photoperiod for 14 days. Another culturing method was to plate single conidia on water agar plates and after 24h transfer them to 17.7% V8 juice agar for two weeks (Steffenson & Webster, 1992). Afanasenko *et al.* (2007) cultured single-conidia isolates of *P. teres* on modified Chapek's medium with lactose and urea (CLM) for 10 days at 20-22°C, under constant light, to obtain cultures for inoculation. Other than V8 Agar and CLM, Potato Dextrose Agar (PDA) and Oatmeal Agar (OMA) have been used for conidia production. To increase the production of conidia for population 37 different media were tested, including Sach's medium, PDA and 50% strength PDA. Preliminary results suggest that the Sach's medium produced more conidia than the PDA agar. However, this observation needs to be further investigated.

4 Chapter 4: Fine mapping QTL region associated with the virulence genes of *P. teres* f. *teres*

4.1 Introduction

Interactions between host resistance and pathogen virulence differ depending on the metabolic pathway through which the resistance is induced. Cloning of the virulence genes will facilitate a better understanding of the interactions between the host and the fungus. To enable the cloning of virulence genes, fine-mapping of the genomic region containing the virulence gene is essential. The aim was thus to fine-map a QTL region identified in the NB029/NB085 population consisting of 83 individuals. A genetic map consisting of DArT-Seq markers of this population has previously been constructed and QTL analysis performed by Dr Anke Martin, Centre for Crop Health, USQ. A QTL associated with virulence was identified on chromosome 1 (Fig. 4.1). This QTL region was identified in a *Pyrenophora teres* f. *teres* genome assembly by Dr Simon Ellwood, Curtin University, Western Australia (Ellwood *et al.*, 2010) who designed primers for SSRs located in this region. These SSR markers were tested in this study to fine-map the QTL region.

HYPOTHESIS:

SSR markers can be used to fine-map QTL region associated with virulence in P. teres f. teres.

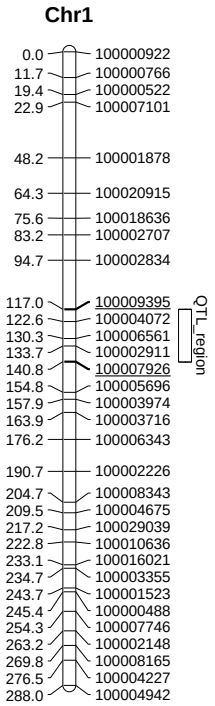


Fig. 4.1 Genetic map of linkage group 1 QTL position is indicated. Flanking markers are underlined

4.2 Materials and methods

4.2.1 DNA extraction

Single-spore cultures of isolates NB029, NB085 and their 83 progeny were grown on PDA plates at 22°C for at least ten days before DNA extraction. Fungal mycelium was scraped from each isolate/hybrid using a sterile blade and placed in a 1.5 ml microcentrifuge tubes containing two metal beads. DNA was extracted using a Wizard Genomic DNA purification kit (Promega Corporation, Sydney, Australia). A volume of 600 µl of Nuclei Lysis Solution was added to each tube and samples homogenised for 15 s at 6m.p.s using a FastPrep (MP Biomedicals, Australia). Sample tubes were incubated for 15 min at 65°C and cooled at room temperature for five minutes before adding 200 µl of Protein Precipitation Solution to each tube. Tubes were vortexed manually for 20 sec each and then centrifuged for 6 min until a tight pellet had formed at the bottom of each tube. Using a micropipette, 700 µl of the supernatant containing the DNA was transferred to a new tube containing 600 µl isopropanol. The tubes containing the supernatant/DNA and the Isopropanol were centrifuged for three min at room temperature. The supernatant was then discarded by draining the tubes gently and 600 µl of

70% ethanol was added. The tubes were again centrifuged at room temperature for two min. The ethanol was decanted and the tubes were left inverted to dry for 15 min before rehydrating with 80 µl sterile distilled water. The concentration of the extracted DNA was quantified with an Implen Nano Photometer (Integrated Sciences, Sydney, Australia).

4.2.2 SSR markers

Primer sequences were obtained from Dr Simon R. Ellwood (Department of Environment and Agriculture, Curtin University, Australia) (Table 4.1). The markers were designed to be in a size range between 200 and 400 base pairs (bps). For carrying out the polymerase chain reaction (PCR), the reaction mixture consisted of 20 ng DNA, 5 µM of each primer, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 1 × buffer (Bioline Pty Ltd., Australia) and 0.1 U Immolase™ DNA polymerase (Bioline Pty Ltd., Australia) in a total volume of 10 µl. The following PCR cycle profile was used: 7 min at 95 °C, followed by 35 cycles at 94 °C for 30 s, 50–60 °C (depending on annealing temperature) for 30 s and 72 °C for 30 s and one cycle at 72 °C for 10 min. The amplified products were visualised using a Gel-Scan 2000™ (Corbett Life Sciences, Sydney, Australia).

Table 4.1 Primer sequences for SSR markers used to fine-map QTL region. The expected fragment size in bps for each maker is given.

Label	Sequence	Length (bp)
0038_5_F	GCATCCAGCACGTAGCAAGTA	240
0038_5_R	CCAAGCCGGTCTCGAAGTAGTA	
0038_6a_F	GCCTCATCCAGGTAAGTACATTACG	250
0038_6a_R	GATTCTTAGCATCTACATCGAGTGC	
0038_6b_F	TTCCAGATGGGTATCGGATTCA	250
0038_6b_R	TATGTCGATTAGATATCGCAATTCG	
0038_7_F	AGAATCTTGCGGTGACGCTT	321
0038_7_R	TAGCTGCGCTGCCGAATG	
0038_8_F	GTACCGAAGACGTCGAAGAGGA	210

0038_8_R	AATGACGTAGATAACTAAACCCGCG	
0038_9_F	ACTGGTCGAGGTCGAGGCG	289
0038_9_R	ATACAATGTGCACGTCGATGCT	
0038_10_F	ATACCAGGAAGGAACACCAAGAC	302
0038_10_R	CCGGTACAAACTCGATACCTAGAA	
0038_11_F	GACCGTAATGGACACGTTTGT	207
0038_11_R	GTTGCTTCATCCAGGTTCGTAT	
0038_12_F	GCACTTAGCGTGGTGTCAGATAGA	268
0038_12_R	GAGTATAGAGGCTCTTAGTACCTCTTAGGTATAC	
0039_6_F	CAGTAAGATTATTCTGTTCTACCTTGC	319
0039_6_R	TTGGTCTCGATCCGATAATCTCTAG	
0039_7_F	CATGTTGAGAAGACGACTTGGTAT	330
0039_7_R	TGGAATGGGCAATGGTTGTAG	
0039_8_F	CTACCGTTGTCTTCAATGCAGATAC	244
0039_8_R	GCTTTCTGGAAGACGCAGGT	
0039_9_F	CATCTGTTACAAACACCATTGCG	257
0039_9_R	TGACCGACTATTCTTCTGAACCAT	
0039_10_F	CGTAGGTGGTTTGATGTCTGTCG	350
0039_10_R	AGGAGGATGTTCTGATGGATGAT	
0039_11_F	CCTGTACCGACATAATAATACACGA	285
0039_11_R	GTGCGTCAGTTTAGCAGCAG	
0039_12_F	CCATATCAACCCAACCTAATCTGT	311
0039_12_R	GGTTCTGATGCAATCTGTGTATGT	

4.2.3 High Resolution Melt Assay - HRM™

HRM analysis was used to detect polymorphism that could not be detected through regular gel electrophoresis.

HRM analysis was performed using a Rotor-Gene™ 6000 (Corbett Life Science, Sydney, Australia). The SSR primers listed above (Table 4.2) were used. The PCR reaction mixture consisted of 20 ng DNA, 1 µM of each primer, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 1 × buffer (Bioline Pty Ltd., Australia), 0.25 U Immolase™ DNA polymerase (Bioline Pty Ltd., Australia) and 0.75 µl (18.75 µM) SYTO®9 (Invitrogen Pty Ltd., Australia) in a total volume of 20 µl. The following PCR cycle profile was used: 7 min at 95 °C, followed by 40 cycles at 95 °C for 15 s, 50–60 °C (depending on annealing temperature) for 20 s and 72 °C for 20. To perform the melt analysis, the temperature was raised by 0.1° each step with continuous attainment of fluorescence to increase from 75°C to 95°C. This was performed directly after the amplification had taken place. To determine the genotypes of each individual line, an automated genotype calling software (Corbett Life Science, version 1.7) was used. According to the protocol provided by the supplier (Corbett Life Science) the HRM analysis was carried out by normalizing the fluorescence versus temperature graphs to 100 to allow all the curves to be compared, and as such to maintain the same starting and ending fluorescent signal level. The regions of normalization were then adjusted using the raw data graph.

4.2.4 Genetic mapping

Using the gel electrophoresis result, a spreadsheet was created scoring the progeny as A or B, NB029 and NB085, respectively. Progeny which did not fall clearly into either class were entered as missing. The Excel spreadsheet was then imported by the Map Manager QTXb20. The genotypic data were assigned as double haploid since the program does not have an option for haploid species and thus the option closest to haploid was chosen. Using the "tools" option in Map Manager QTXb20, the marker was added to the most suitable position. The marker was then ordered manually through the "Find – report links" between the markers with the highest logarithm of odds (LOD) score, 23.8 to estimate the likelihood of two loci linkage point.

4.3 Results

To fine-map the QTL region 16 SSR makers were tested across the parents NB029 and NB085 to identify polymorphic makers (Fig. 4.2). Out of the 16 SSR markers screened only one (0038_10) was polymorphic between the parents (Fig. 4.3).

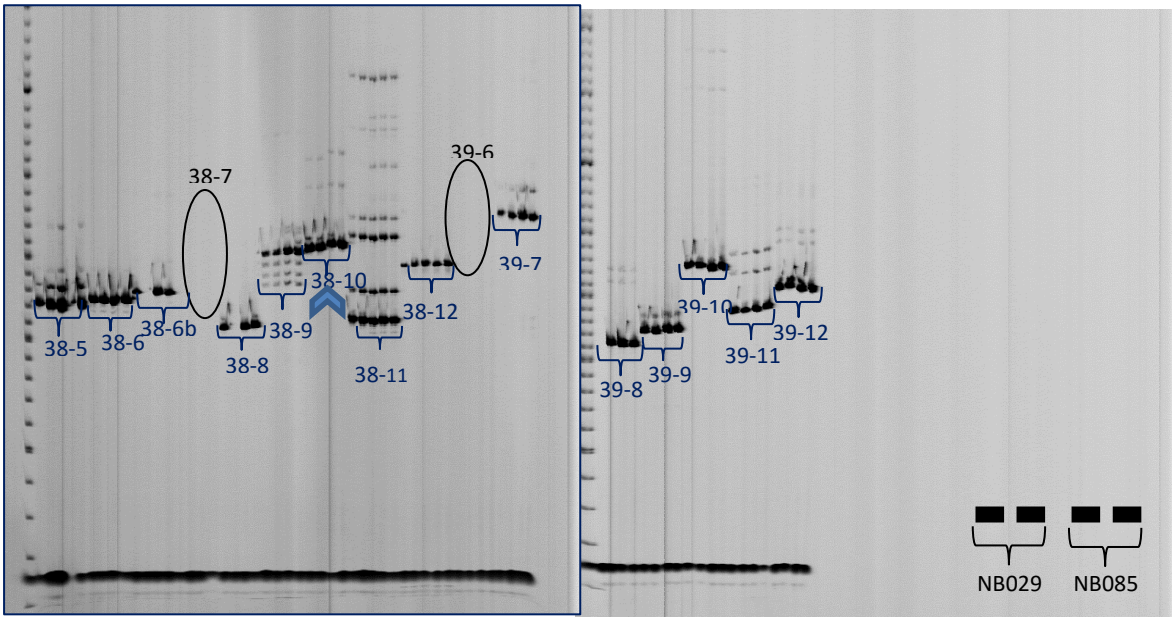


Fig. 4.2 Sixteen SSR markers were screened over two replicates of each of the parents NB029 and NB085 to detect polymorphism. The circled markers could not be amplified even through different annealing temperatures of 50, 55 and 60°C were used. The arrow indicates the polymorphic marker that was mapped in the QTL region. The rest of markers were not polymorphic but were re-tested using the HRM™.

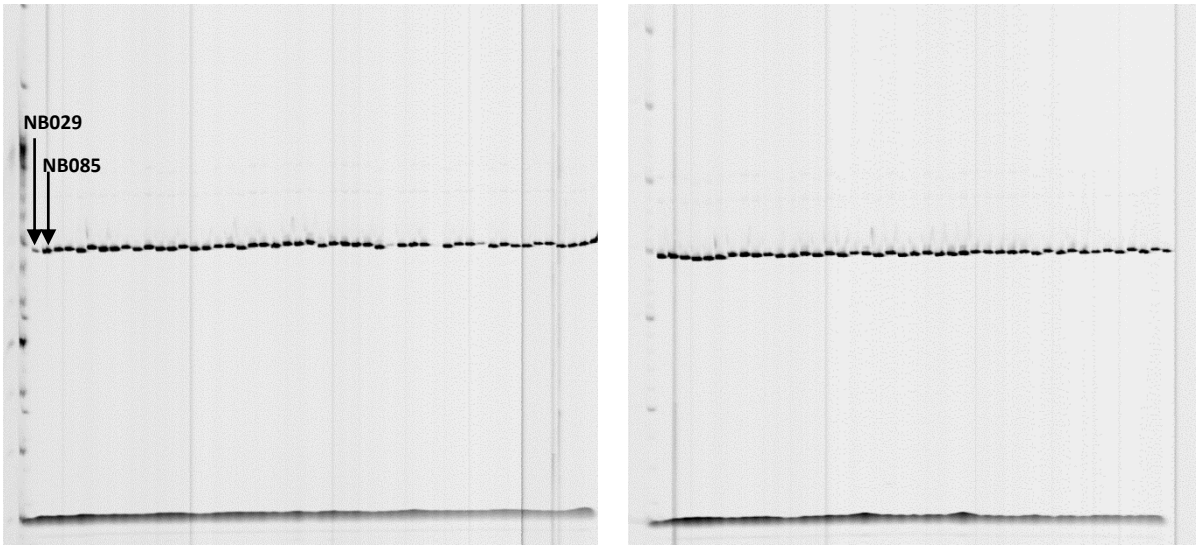


Fig.4.3 SSR marker 0038_10 screened over population NB029/NB085. The marker shows polymorphism between the progeny and so was mapped to chromosome 1 in the QTL region and co-located with DArT-seq marker 100006561.

The polymorphic marker was amplified across DNA of the lines of the NB029/NB085 population. The marker was then mapped to chromosome 1 in the QTL region (Fig. 4.4) and was co-located with DArT-seq marker 100006561.

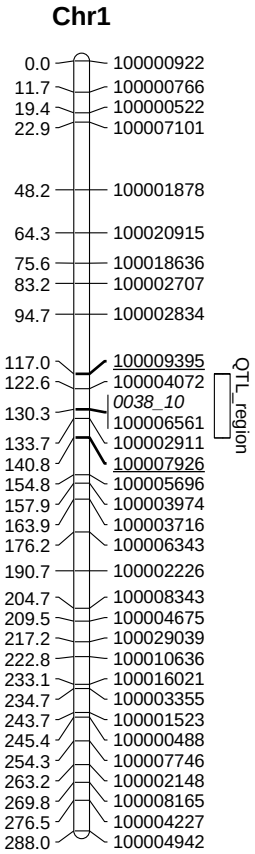


Fig. 4.4 Genetic map of linkage group 1. Underlined markers indicate markers flanking QTL. SSR marker added to the map is indicated in italics.

Using the HRM technique, four copies of the parental DNA, NB029 and NB085 were amplified with the remaining markers. No polymorphism was detected (Fig. 4.5), confirming the results of the gel electrophoresis. However, the polymorphic marker 0038-10 was screened as well using the HRM technique and its polymorphism was confirmed as the melting curve showed two distinctive bands at different melting temperatures TM (Fig. 4.6).

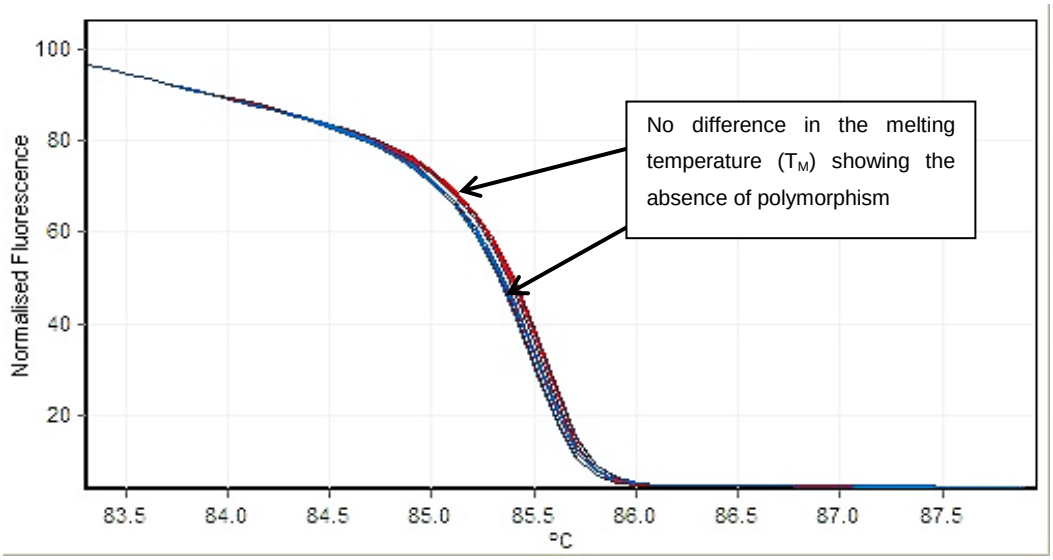


Fig. 4.5 Normalized Graph for HRM _four replicates of NB029 (Red lines) and NB085 (Blue lines) DNA were amplified through the SSR marker 0038-6b

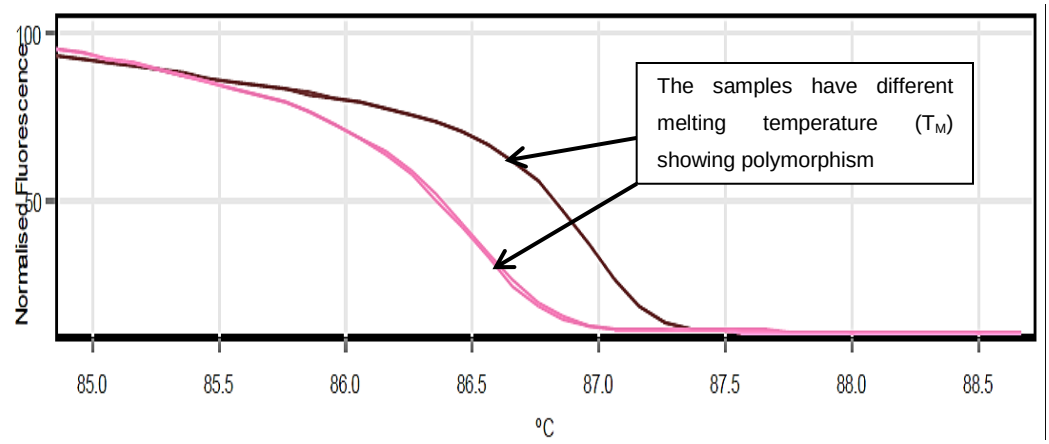


Fig. 4.6 Normalized Graph for HRM _four replicates of NB029 (purple lines) and NB085 (pink lines) DNA were amplified through the SSR polymorphic marker 0038-10

4.4 Discussion:

A DArT-seq genetic map consisting of DArT-Seq markers of *Pyrenophora teres* f. *teres* was constructed by Dr Anke Martin, Centre for Crop Health, USQ. A QTL analysis was performed by Dr Martin and one QTL region associated with *Pyrenophora teres* f. *teres* virulence identified. In this study, more SSR markers were added to this QTL region to help with further identification of potential virulence gene(s) in this region.

To fine map this QTL region, 16 SSR markers were designed by Dr Simon R. Ellwood (Department of Environment and Agriculture, Curtin University, Australia) and were tested for polymorphism across the parents. Only one of these markers was polymorphic across the two parents. This polymorphic marker, 0038_10 was then screened over the DNA of all the progeny, including the parents and gel electrophoresis was used to display the results. The progeny were then genotyped and marker 0038_10 was mapped on chromosome 1 in the QTL region.

Despite the expansion of using molecular markers and QTL mapping, most of studies on net blotch were focused on identifying QTL regions associated either with resistance in barley or avirulence in *P. teres*, rather than locating similar regions of virulence on *P. teres*. For example, Lai *et al.* (2007) constructed an AFLP-based linkage map using a cross between two *P. teres* isolates: 15A and 0-1. This cross was used to identify genes associated with avirulence in three different barley lines: Prato, Tifang and Canadian Lake Shore (CLS). The authors concluded that two main genes were responsible for the avirulence of *P. teres* to Prato: *AvrPra1* and *AvrPra2* (Lai *et al.*, 2007). Similarly, Beattie *et al.* (2007) phenotyped the progeny of one avirulent isolate: WRS 1906 with a highly virulent isolate: WRS 1607 over the barley variety Heartland. Using AFLP and BSA, Beattie *et al.* (2007) identified six markers closely linked to a avirulence gene ($Avr_{Heartland}$) in that progeny (Beattie *et al.*, 2007).

Recently, Shjerve *et al.* (2014) used genetic maps generated by SSR, SNP and AFLP markers to locate four virulence QTL regions on *Ptt*, VK1 and VK2, virulent on Kombar and VR1 and VR2, virulent on Rika. In their study,

Shjerve *et al.* (2014) screened a *P. teres* f. *teres* cross 15A x 6A on barley lines Rika and Kombar. However, the main approach of that study was to investigate the *P. teres* f. *teres* – barley interaction in relevance with other studies carried out by the same group to detect resistance QTL in barley.

By adding the marker to the virulence QTL region on *P. teres* f. *teres* genome, we are achieving better knowledge of the genetics of the pathogen. With further fine-mapping and identifying more virulence genes, cloning of those genes would be possible. This will enable us to further investigate the interaction that takes place between those genes and resistance/susceptibility genes in the host.

5 Chapter 5: General discussion and conclusion

5.1 Discussion

Pyrenophora teres is an increasingly damaging pathogen to the barley industry worldwide (Food and Agriculture Organisation, 2005). The occurrence of the sexual stage of *P. teres* in the field makes the appearance of potential new virulences highly possible (Lehmensiek *et al.*, 2010; McLean *et al.*, 2014) . Such novel virulences may be able to infect previously resistant commercial barley lines leading to significant crop losses, not only to the current harvest but to neighbouring fields and future harvest for the three years that follow as the pathogen has the ability to survive in the infested stubble for that period of time (Bretag, 2009) . It has also been documented that *P. teres* has the ability to adapt to different fungicides lowering their effectiveness (Campbell *et al.*, 2002; Serenius & Manninen, 2006) .

To better understand changes in virulence of the pathogen, we tested three hypotheses during the conduct of this study.

The first hypothesis, "*DLA – spray method can replace both seedling assay and DLA – droplet method for phenotyping net blotch of barley"* was validated where a new phenotyping method to screen *P. teres* isolates, including hybrids was developed in this study. This method (DLA – spray method) has the ability to substitute other methods: detached leaf assay – droplet method and seedling assay, as it provides confinement of the hybrid within a controlled environment (laboratory) while it clearly expresses the type of lesion of the sample screened, either spot or net form, and its severity.

The second hypothesis, "*Hybrids of P. teres express virulences different to their parents'"* was also supported. The virulences of hybrids generated in the laboratory on different barley genotypes were investigated. From this investigation it can be concluded that some recombinants and hybrids differ in their virulences from the parental isolates. Besides showing different reactions to different barley genotypes, it was more interesting to find out

how some of those hybrids were either totally avirulent or virulent to most of the genotypes unlike either of the parents. Such an outcome can indicate clearly the anticipated economic loss upon the occurrence of such virulent hybrids in the field during the absence of resistant barley varieties.

The third hypothesis "*SSR markers can be used to fine-map QTL region associated with virulence in P. teres f. teres"* was validated as well. Fine-mapping was undertaken to add markers to a QTL region associated with virulence in a NB029 x NB085 isolate cross. A new marker was added to this QTL region.

5.2 Conclusion

The most appropriate net blotch disease management technique is to use resistant varieties. Yet, developing such varieties is not easy as strong and valid sets of phenotypic data are needed to analyse both, the host-pathogen genetic interaction and the diverse nature of this pathogen as well. To obtain such data, the new phenotyping method we developed represents a cheaper, less space requiring substitute compared to the other phenotyping method including seedling assay and the phytotoxin assay. This method also offers safer investigation of the laboratory generated hybrids rather than the seedling assay since all the isolates, either of known or unknown virulences are confined in the tray. Furthermore, in the DLA spray method, an accurate and specific identification of the hybrid lesion type can be achieved which is not the case for the DLA - droplet method.

A key to the threat of that pathogen is its heterothallism that results in recombination and new virulences. Recombination between the two forms results in hybrids that may be more virulent than the parents, which was observed in this study as hybrids 26.10, 26.11 and 26.14 all were more virulent than both of the parents. The occurrence of such hybrids has rarely been documented. Possibly this is not due to their generation being a rare event but due to the fact that it is difficult for pathologists and researchers to detect the presence of a hybrid in the field since the majority of hybrids produce lesions that are either spot form or net form in their appearance. In order to unequivocally identify hybrids a combination of genetic analysis such

as AFLP markers, and screening across multiple host lines would be required to identify hybrid progeny in the field.

Through investigating the behaviour of hybrids of crosses generated in the laboratory at USQ, it becomes clear that only 10% of the hybrids tested showed intermediate symptoms where some of the varieties showed spot type lesions while other varieties inoculated with the same inoculum showed net type lesions. Furthermore, the hybrids also have their own pattern of virulence with some being totally avirulent compared to the parents and other such as hybrid 26.10 showing high virulence even against some resistant genotypes supporting the second hypothesis regarding the different behaviour between parents and hybrids of *P. teres*. Hybrid avirulence may be due to recombination of virulence/avirulence genes (Arabi *et al.*, 2003) or it may reflect a loss of fitness generally in some hybrid progeny. Such lines, while observable in the laboratory may not be obvious in the field due to poor competitiveness and survival or due to the presence of other diseases and/or factors that cause similar foliar symptoms such as *Cochliobolus sativus* the causal agent of spot blotch or boron toxicity (Campbell *et al.*, 2002).

In order to better understand the host-pathogen genetic interaction, we fine-mapped a QTL region in the pathogen associated with its virulence. Unfortunately this region was not highly polymorphic between the two parents, with only one of 16 markers segregating in the population. Using SSR markers, a new marker was added to the existing genetic map of the NB29/NB85 *P. teres* f. *teres* cross. This approach can be applied in future studies of other virulence genes.

5.3 Future prospects

Many studies were carried out previously based on laboratory generated hybrids of *P. teres* and more are anticipated due to the evident occurrence of hybridization of *P. teres* in the field (McLean *et al.*, 2014) . Now that this study has proved the reliability and accuracy of the DLA – spray method as a new phenotyping method of net blotch of barley, it would be a suitable substitute for other methods to study hybrids of *P. teres*. The main advantage of this new method is its ability to display the lesion type of the hybrid which

leads to identifying its type, SFNB or NFNB while confining the isolates under quarantine conditions.

With the availability of whole genomic maps of both *P. teres* and barley, and considering the rapid development and availability of molecular techniques, future studies can achieve crucial results in regards to developing new resistant barley varieties. The main direction recently is the use of map-based methods to clone virulence genes, which can be very informative once resistance genes in the host and virulence genes in the pathogen are tagged and identified (Lu *et al.*, 2012; Oliver & Osbourn, 1995; Weiland *et al.*, 1999) .

It would be highly recommended to pursue investigations that further exploit available resources such as the first genome assembly of *P. teres* carried out by Ellwood *et al.* (2010). In their study, Ellwood *et al.* (2010) suggest that their map would be used to characterize and isolate both virulence and avirulence associated genes through map-based cloning. An approach that we tried to achieve in this study as we did tag a gene associated with the virulence of *P. teres* f. *teres*. With the addition of further markers to the QTL region, those genes should be isolated and cloned to study their interaction with elected barley genotypes.

Once those genes are thoroughly studied, a following step would be to use targeted gene disruption to develop defective mutants only in the virulence genes (Oliver & Osbourn, 1995) . This approach has previously been applied to a study of *PTK1* which is responsible for appressoria formation in *P. teres* (Ruiz-Roldán *et al.*, 2001). These authors successfully developed mutants of *P. teres* carrying disrupted genes. When tested, those mutants had no ability to either infect barley or to colonize wounded tissues.

Through this study, certain hybrids have shown complete avirulence to all the tested genotypes. Using those avirulent isolates and the *P. teres* map carried out by Ellwood *et al.* (2010), a QTL associated with the avirulence of *P. teres* can be identified to tag avirulence genes. Detecting avirulence genes can be as valuable as the identification of virulence genes in *P. teres*. Using map based cloning (De Wit, 1995; Knogge, 1996) ; we will be able to understand the nature and characters of race specific elicitors which are the avirulence

genes products recognized by the host resistance genes (De Wit, 1997; Shjerve *et al.*, 2014) . Following the gene-for-gene hypothesis (Flor, 1956) , the avirulence genes in a pathogen mostly have corresponding resistant genes in the host. According to this theory, future studies can have two directions, the first one is to use isolated and cloned avirulence genes from avirulent *P. teres* isolates to introduce mutated avirulent individuals to the field. The second direction is to develop new transgenic resistant barley lines using map-based cloning of both, avirulence genes of *P. teres* and resistance genes of barley (De Wit, 1997; Lai *et al.*, 2007; Manninen *et al.*, 2000).

References

Abu Qamar, M., Liu, Z. H., Faris, J. D., Chao, S., Edwards, M. C., Lai, Z., Franckowiak, J. D., & Friesen, T. L. (2008). A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. *Theoretical and Applied Genetics, 117*(8), 1261-1270. doi: 10.1007/s00122-008-0860-x

Afanasenko, O., Mironenko, N., Filatova, O., Kopahnke, D., Krämer, I., & Ordon, F. (2007). Genetics of host-pathogen interactions in the *Pyrenophora teres* f. *teres* (net form) – barley (*Hordeum vulgare*) pathosystem. *European Journal of Plant Pathology, 117*(3), 267-280. doi: 10.1007/s10658-006-9093-5

Arabi, M. I., Barrault, G., Sarrafi, A., & Albertini, L. (1992). Variation in the resistance of barley cultivars and in the pathogenicity of *Drechslera teres* f. sp. *maculata* and *D. teres* f. sp. *teres* isolates from France. *Plant Pathology, 41*, 180-186. doi: 10.1111/j.1365-3059.1992.tb02336.x

Arabi, M. I. E., Al-Safadi, B., & Charbaji, T. (2003). Pathogenic variation among isolates of *Pyrenophora teres*, the causal agent of barley net blotch. *Journal of Phytopathology, 151*, 376-382. doi: 10.1046/j.1439-0434.2003.00734.x

Atanasoff, D. (1920). *Fusarium*-blight (scab) of wheat and other cereals. *Journal of Agricultural Research, 20*(1), 1-32.

Bach, E., Christensen, S., Dalgaard, L., Larsen, P. O., Olsen, C. E., & Smedegård-Petersen, V. (1979). Structures, properties and relationship to the aspergillomarasmines of toxins produced by *Pyrenophora teres*. *Physiological Plant Pathology, 14*, 41-46. doi: 10.1016/0048-4059(79)90023-7

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., & Parr-Dobrzanski, B. (2002). The strobilurin fungicides. *Pest Management Science, 58*(7), 649-662.

Beattie, A. D., Scoles, G. J., & Rossnagel, B. G. (2007). Identification of molecular markers linked to a *Pyrenophora teres* avirulence gene. *Phytopathology, 97*(7), 842-849. doi: 10.1094/PHYTO-97-7-0842

Becker, J., & Heun, M. (1995). Barley microsatellites: allele variation and mapping. *Plant Molecular Biology, 27*(4), 835-845. doi: 10.1007/BF00020238

Bockelman, H. E., Sharp, E. L., & Eslick, R. F. (1977). Trisomic analysis of genes for resistance to scald and net blotch in several barley cultivars. *Canadian Journal of Botany, 55*(15), 2142-2148. doi: 10.1139/b77-242

Bretag, T. (2009). Net form of net blotch (exotic pathotypes) *Pyrenophora teres* f. sp. *teres Plant Health Australia*. Australia.

Brooks, F. E. (2008). Detached-leaf bioassay for evaluating taro resistance to *Phytophthora colocasiae*. *Plant Disease, 92*, 126-131. doi: 10.1094/PDIS-92-1-0126

Browne, R. A., & Cooke, B. M. (2005). Resistance of wheat to *Fusarium* spp. in an in vitro seed germination assay and preliminary investigations into the relationship with Fusarium head blight resistance. *Euphytica, 141*(1-2), 23-32. doi: 10.1007/s10681-005-4820-0

Buchannon, K., & Wallace, H. (1962). Note on the effect of leaf diseases on yield, bushel weight and thousand-kernel weight of Parkland barley. *Canadian Journal of Plant Science, 42*(3), 534-536. doi: 10.4141/cjps62-085

Caeseele, L. V., & Grumbles, J. (1979). Ultrastructure of the interaction between *Pyrenophora teres* and a susceptible barley host. *Canadian Journal of Botany, 57*, 40-47. doi: 10.1139/b79-009

Cakir, M., Gupta, S., Platz, G. J., Ablett, G. A., Loughman, R., Emebiri, L. C., Poulsen, D., Li, C. D., Lance, R. C. M., Galwey, N. W., Jones, M. G. K., & Appels, R. (2003). Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.). *Australian Journal of Agricultural Research, 54*, 1369-1377. doi: 10.1071/. AR01027

Campbell, G. F., & Crous, P. W. (2003). Genetic stability of net × spot hybrid progeny of the barley pathogen *Pyrenophora teres*. *Australasian Plant Pathology, 32*, 283-287. doi: 10.1071/AP03016

Campbell, G. F., Crous, P. W., & Lucas, J. A. (1999). *Pyrenophora teres* f. *maculata*, the cause of *Pyrenophora* leaf spot of barley in South Africa. *Mycological Research, 103*(3), 257-267. doi: 10.1017/S0953756298007114

Campbell, G. F., Lucas, J. A., & Crous, P. W. (2002). Evidence of recombination between net- and spot-type populations of *Pyrenophora teres* as determined by RAPD analysis. *Mycological Research, 106*(5), 602-608. doi: 10.1017/s0953756202005853

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica, 142*(1-2), 169-196. doi: 10.1007/s10681-005-1681-5

Crous, P. W., Janse, B. J. H., Tubridge, J., & Holz, G. (1995). DNA homology between *Pyrenophora japonica* and *P. teres*. *Mycological Research, 99*(9), 1098-1102. doi: 10.1016/S0953-7562(09)80778-8

Cruz, V. M., Kilian, A., & Dierig, D. A. (2013). Development of DArT marker platforms and genetic diversity assessment of the US collection of the new oilseed crop *lesquerella* and related species. *PLoS One, 8*, 1-13. doi: 10.1371/journal.pone.0064062

Davierwala, A., Chowdari, K., Kumar, S., Reddy, A., Ranjekar, P., & Gupta, V. (2000). Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica, 108*(3), 269-284. doi: 10.1023/A:1004160232679

De Wit, P. J. G. M. (1995). Fungal avirulence genes and plant resistance genes: unraveling the molecular basis of gene-for-gene interactions. *Advances in Botanical Research, 21*, 148-186. doi: 10.1016/S0065-2296(08)60012-9

De Wit, P. J. G. M. (1997). Pathogen avirulence and plant resistance: A key role for recognition. *Trends in Plant Science, 2*. doi: 10.1016/S1360-1385(97)01139-4

Deadman, M., & Cooke, B. (1985). A method of spore production for *Drechslera teres* using detached barley leaves. *Transactions of the British Mycological Society, 85*(3), 489-493. doi: 10.1016/S0007-1536(85)80045-0

Dilger, M., Felsenstein, F. G., & Schwarz, G. (2003). Identification and quantitative expression analysis of genes that are differentially expressed during conidial germination in *Pyrenophora teres*. *Molecular Genetics and Genomics, 270*(2), 147-155. doi: 10.1007/s00438-003-0910-7

Drechsler, C. (1923). Some graminicolons species of *Helminthosporium. I. Journal of Agricultural Research, 24*(8), 641-739

Ellwood, S. R., Liu, Z., Syme, R. A., Lai, Z., Hane, J. K., Keiper, F., Moffat, C. S., Oliver, R. P., & Friesen, T. L. (2010). A first genome assembly of the barley

fungal pathogen *Pyrenophora teres* f. *teres*. *Genome Biol, 11*(11), 1-14. doi: 10.1186/gb-2010-11-11-r109

Emebiri, L. C., Platz, G., & Moody, D. B. (2005). Disease resistance genes in a doubled haploid population of two-rowed barley segregating for malting quality attributes. *Australian Journal of Agricultural Research, 56*(1), 49. doi: 10.1071/ar04102

Flor, H. H. (1956). The complementary genec systems in flax and flax rust. *Advances in Genetics Incorporating Molecular Genetic Medicine, 8*, 29-54. doi: 10.1016/S0065-2660(08)60498-8

Food and Agriculture Organisation. (2005). Food and agricultural commodities production. Retrieved 23rd October, 2006, from http://www.fao.org/

Friesen, T. L., Ali, S., Stack, R. W., Francl, L. J., & Rasmussen, J. B. (2003). Rapid and efficient production of the *Pyrenophora tritici-repentis* teleomorph. *Canadian Journal of Botany, 81*(8), 890-895. doi: 10.1139/b03-082

Friesen, T. L., Faris, J. D., Lai, Z., & Steffenson, B. J. (2006). Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. *Genome, 49*(7), 855-859. doi: 10.1139/g06-024

Friis, P., Olsen, C. E., & Møller, B. L. (1991). Toxin production in *Pyrenophora teres*, the ascomycete causing the net-spot blotch disease of barley (*Hordeum vulgare* L.). *The Journal of Biological Chemistry, 266*, 13329-13335. doi: 10.1111/j.1364-3703.2010.00649.x

Gallagher, J., Biscoe, P., & Scott, R. (1975). Barley and its environment. V. stability of grain weight. *Journal of Applied Ecology*, 319-336. doi: 10.2307/2401735

Grewal, T. S., Rossnagel, B. G., Pozniak, C. J., & Scoles, G. J. (2008). Mapping quantitative trait loci associated with barley net blotch resistance. *Theoretical and Applied Genetics, 116*(4), 529-539. doi: 10.1007/s00122-007-0688-9

Gupta, S., Li, C., Loughman, R., Cakir, M., Westcott, S., & Lance, R. (2011a). Identifying genetic complexity of 6H locus in barley conferring resistance to *Pyrenophora teres* f. *teres*. *Plant Breeding, 130*(4), 423-429. doi: 10.1111/j.1439-0523.2011.01854.x

Gupta, S., Li, C. D., Loughman, R., Cakir, M., Platz, G., Westcott, S., Bradley, J., Broughton, S., & Lance, R. (2010a). Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (*Pyrenophora teres* f. *teres*) isolates. *Plant Breeding, 129*, 362—368. doi: 10.1111/j.1439-0523.2009.01716.x

Gupta, S., Li, C. D., Loughman, R., Cakir, M., Platz, G., Westcott, S., Bradley, J., Broughton, S., & Lance, R. (2010b). Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (*Pyrenophora teres* f. *teres*) isolates. *Plant Breeding, 129*, 362-368. doi: 10.1111/j.1439-0523.2009.01716.x

Gupta, S., Loughman, R., D'Antuono, M., & Bradley, J. (2011b). Characterisation and diversity of *Pyrenophora teres* f. *maculata* isolates in Western Australia. *Australasian Plant Pathology, 41*(1), 31-40. doi: 10.1007/s13313-011-0083-5

Hamada, H., Petrino, M. G., & Kakunaga, T. (1982). A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proceedings of the National Academy of Sciences, 79*(21), 6465-6469. doi: 10.1073/pnas.79.21.6465

Hammond-Kosack, K. E., & Jones, J. D. (1996). Resistance gene-dependent plant defense responses. *The Plant Cell, 8*(10), 1773-1791. doi: 10.1105/tpc.8.10.1773

Hebert, T. T. (1971). The perfect stage of *Pyricularia grisea*. *Phytopathology, 61*, 83-87. doi: 10.1094/Phyto-61-83

Ho, K. M., Tekauz, A., Choo, T. M., & Martin, R. A. (1996). Genetic studies on net blotch resistance in a barley cross. *Canadian Journal of Plant Science, 76*, 715-719. doi: 10.4141/cjps96-123

Hochman, Z., Gobbett, D., Holzworth, D., McClelland, T., van Rees, H., Marinoni, O., Garciaa, J. N., & Horan, H. (2012). Quantifying yield gaps in rainfed cropping systems: A case study of wheat in Australia. *Field Crops Research, 136*, 85-96. doi: 10.1016/j.fcr.2012.07.008

. HRM Assay Design and Analysis CorProtocol™ 6000. (2006). In C. Research (Ed.).

Ismail, I. A., Godfrey, D., & Able, A. J. (2014). Fungal growth, proteinaceous toxins and virulence of *Pyrenophora teres* f. *teres* on barley. *Australasian Plant Pathology, 43*(5), 535-546. doi: 10.1007/s13313-014-0295-6

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research, 29*(4), 1-7. doi: 10.1093/nar/29.4.e25

Jayasena, K., Loughman, R., & Majewski, J. (2002). Evaluation of fungicides in control of spot-type net blotch on barley. *Crop Protection, 21*(1), 63-69. doi: 10.1016/S0261-2194(01)00118-1

Johnsson, L., Hökeberg, M., & Gerhardson, B. (1998). Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against cereal seed-borne diseases in field experiments. *European Journal of Plant Pathology, 104*(7), 701-711. doi: 10.1023/A:1008632102747

Jones, C. J., Edwards, K. J., Castaglione, S., Winfield, M. O., Sala, F., Van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A., & Karp, A. (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding, 3*(5), 381-390. doi: 10.1023/A:1009612517139

Jordan, V. W. L. (1981). Aetiology of barley net blotch caused by *Pyrenophora teres* and some effects on yield. *Plant Pathology, 30*, 77-87. doi: 10.1111/j.1365-3059.1981.tb01232.x

Jordan, V. W. L., & Allen, E. C. (1984). Barley net blotch: influence of straw disposal and cultivation methods on inoculum potential, and on incidence and severity of autumn disease. *Plant Pathology, 33*(4), 547-559. doi: 10.1111/j.1365-3059.1984.tb02879.x

Jordan, V. W. L., Best, G. R., & Allen, E. C. (1985). Effects of *Pyrenophora teres* on dry matter production and yield components of winter barley. *Plant Pathology, 34*, 200-206. doi: 10.1111/j.1365-3059.1985.tb01350.x

Jørgensen, H. J. L., Lübeck, P. S., Thordal-Christensen, H., de Neergaard, E., & Smedegård-Petersen, V. (1998). Mechanisms of induced resistance in barley against *Drechslera teres*. *Phytopathology, 88*, 698-707. doi: 10.1094/PHYTO.1998.88.7.698

Kanchanaraksa, S. (2008). Evaluation of diagnostic and screening tests: Validity and reliability Baltimore: John Hopkins University

Kattan, M. W., & Cowen, M. E. (2009). *Encyclopedia of medical decision making* (Vol. 1). Thousand Oaks, California, USA: Sage Publications Inc.

Kenneth, R. (1962). On the taxonomy, morphology and geographic origins of *Pyrenophora teres* Drechsler and allied species. *Bulletin of the Research Council of Israel, 11*, 55-82.

Kenneth, R. (1964). Conidial release in some *Helminthosporia*. *Nature, 202*, 1025-1026. doi: 10.1038/2021025a0

Keon, J. P. R., & Hargreaves, J. A. (1983). A cytological study of the net blotch disease of barley caused by *Pyrenophora teres*. *Physiological Plant Pathology, 22*, 321-329. doi: 10.1016/S0048-4059(83)81019-4

Khan, T. (1989). Effect of spot-type net blotch (*Drechslera teres* (Sacc.) Shoem) infection on barley yield in short season environment of northern cereal belt of Western Australia. *Crop and Pasture Science, 40*(4), 745-752. doi: 10.1071/AR9890745

Khan, T. N. (1987). Relationship between net blotch (*Drechslera teres*) and losses in grain yield of barley in Western Australia. *Australian Journal of Agricultural Research, 38*, 671-679. doi: 10.1071/AR9870671

Khan, T. N., & Boyd, W. J. R. (1969). Environmentally induced variability in the host reaction of barley to net blotch. *Australian Journal of Biological Science, 22*, 1237-1244. doi: 10.1071/BI9691237

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., Hopper, C., Aschenbrenner-Kilian, M., Evers, M., Peng, K., Cayla, C., Hok , P., & Uszynski, G. (2012). Diversity arrays technology: a generic genome profiling technology on open platforms *Data Production and Analysis in Population Genomics: Methods and Protocols* (Vol. 888, pp. 67-89): Humana Press.

Knight, N. L., & Sutherland, M. W. (2013). Histopathological assessment of wheat seedling tissues infected by *Fusarium pseudograminearum*. *Plant Pathology, 62*, 679-687. doi: 10.1111/j.1365-3059.2012.02663.x

Knogge, W. (1996). Molecular basis of specificity in host/fungus interactions. *European Journal of Plant Pathology, 102*(9), 807-816. doi: 10.1007/BF01877049

König, J., Perovic, D., Kopahnke, D., & Ordon, F. (2013). Development of an efficient method for assessing resistance to the net type of net blotch (*Pyrenophora teres* f. *teres*) in winter barley and mapping of quantitative trait loci for resistance. *Molecular Breeding, 32*(3), 641-650. doi: 10.1007/s11032-013-9897-x

König, J., Perovic, D., Kopahnke, D., Ordon, F., & Léon, J. (2014). Mapping seedling resistance to net form of net blotch (*Pyrenophora teres* f. *teres*) in barley using detached leaf assay. *Plant Breeding, 133*(3), 356-365. doi: 10.1111/pbr.12147

Kronstad, J. W., & Staben, C. (1997). Mating type in filamentous fungi *Annual Review Genetics, 31*, 245-276. doi: 10.1146/annurev.genet.31.1.245

Kumar, K., Xi, K., Turkington, T. K., Tekauz, A., Helm, J. H., & Tewari, J. P. (2011). Evaluation of a detached leaf assay to measure *Fusarium* head blight resistance components in barley. *Canadian Journal of Plant Pathology, 33*(3), 364-374. doi: 10.1080/07060661.2011.590820

Lai, Z., Faris, J. D., Weiland, J. J., Steffenson, B. J., & Friesen, T. L. (2007). Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. *Fungal Genetics and Biology, 44*(5), 323-329. doi: 10.1016/j.fgb.2006.11.009

Lehmensiek, A., Sutherland, M. W., & McNamara, R. B. (2008). The use of high resolution melting (HRM) to map single nucleotide polymorphism markers linked to a covered smut resistance gene in barley. *Theoretical and Applied Genetics, 117*(5), 721-728. doi: 10.1007/s00122-008-0813-4

Lehmensiek, A. M., Bester-van der Merwe, A. E., Sutherland, M. W., Platz, G. J., Krield, W. M., Potgietere, G. F., & Prins, R. (2010). Population structure of South African and Australian *Pyrenophora teres* isolates. *Plant Pathology, 59*(3), 504-515. doi: 10.1111/j.1365-3059.2009.02231.x

Leisova, L., Minarikova, V., Kucera, L., & Ovesna, J. (2005). Genetic diversity of *Pyrenophora teres* isolates as detected by AFLP analysis. *Phytopathology, 153*(10), 569-578. doi: 10.1111/j.1439-0434.2005.01019.x

Leisova, L., Minarikova, V., Kucera, L., & Ovesna, J. (2006). Quantification of *Pyrenophora teres* in infected barley leaves using real-time PCR. *J Microbiol Methods, 67*(3), 446-455. doi: 10.1016/j.mimet.2006.04.018

Leonard, W. H., & Martin, J. H. (1963). Cereal crops. *The Macmillan Company, New York*.

Lightfoot, D. J., & Able, A. J. (2010). Growth of *Pyrenophora teres* in planta during barley net blotch disease. *Australasian Plant Pathology, 39*, 499-507. doi: 10.1071/AP10121

Liu, Z. W., Biyashev, R., & Maroof, M. S. (1996). Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theoretical and Applied Genetics, 93*(5-6), 869-876. doi: 10.1007/BF00224088

Liu, Z. W., Ellwood, S. R., Oliver, R. P., & Friesen, T. L. (2011). *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. *Mol Plant Pathol, 12*(1), 1-19. doi: 10.1111/j.1364-3703.2010.00649.x

Louw, J. P. J., Crous, P. W., & Holz, G. (1994). *Pyrenophora japonica* occurring on barley in South Africa. *Plant Pathology, 43*, 420-423. doi: 10.1111/j.1365-3059.1994.tb02707.x

Lu, S., Edwards, M. C., & Friesen, T. L. (2012). Genetic variation of single nucleotide polymorphisms identified at the mating type locus correlates with form-specific disease phenotype in the barley net blotch fungus *Pyrenophora teres*. *European Journal of Plant Pathology, 135*(1), 49-65. doi: 10.1007/s10658-012-0064-8

Ma, Z., Lapitan, N. L. V., & Steffenson, B. (2004). QTL mapping of net blotch resistance genes in a doubled-haploid population of six-rowed barley. *Euphytica, 137* 291-296. doi: 10.1023/B:EUPH.0000040441.36990.58

Manninen, O., Kalendar, R., Robinson, J., & Schulman, A. (2000). Application of BARE-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley. *Molecular and General Genetics, 264*(3), 325-334. doi: 10.1007/s004380000326

Manninen, O. M., Jalli, M., Kalendar, R., Schulman, A., Afanasenko, O., & Robinson, J. (2006). Mapping of major spot-type and net-type net-blotch resistance genes in the Ethiopian barley line CI 9819. *Genome, 49*(12), 1564-1571. doi: 10.1139/g06-119

Mathre, D. E. (1997). Compendium of barley diseases [Press release]

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., Huang, N., Ishii, T., & Blair, M. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology, 35*(1-2), 89-99. doi: 10.1023/A:1005711431474

McDonald, W. C. (1963). Heterothallism in *Pyrenophora teres*. *Phytopathology, 53*, 771-773.

McDonald, W. C. (1967). Variability and inheritance of morphological mutants in *Pyrenophora teres*. *Phytopathology, 57*.

McLean, M. S. (2011). *The epidemiology and control of spot form of net blotch of barley in Victoria, Australia.* (Ph.D.), The University of Melbourne, Melbourne, Parkville, Australia. .

McLean, M. S., Howlett, B. J., & Hollaway, G. J. (2009). Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. *Crop and Pasture Science, 60*(4), 303-315. doi: 10.1071/CP08173

McLean, M. S., Keiper, F. J., & Hollaway, G. J. (2010). Genetic and pathogenic diversity in *Pyrenophora teres* f. *maculata* in barley crops of Victoria, Australia. *Australasian Plant Pathology, 39*(7), 319–325. doi: 10.1071/AP09097

McLean, M. S., Martin, A., Gupta, S., Sutherland, M. W., Hollaway, G. J., & Platz, G. J. (2014). Validation of a new spot form of net blotch differential set and evidence for hybridisation between the spot and net forms of net blotch in Australia. *Australasian Plant Pathology, 43*(3), 223-233. doi: 10.1007/s13313-014-0285-8

Mironenko, N. V., Afanasenko, O., Filatova, O., & Kopahnke, D. (2005). Genetic control of virulence of *Pyrenophora teres* Drechs., the causative agent of net blotch in barley. *Russian Journal of Genetics, 41*(12), 1674-1680.

Mode, C. J., & Schaller, C. W. (1958). Two additional factors for host resistance to net blotch in barley. *Agronomy, 50*, 15-18. doi: 10.2134/agronj1958.00021962005000010005x

Murray, G. M., & Brennan, J. P. (2009). The current and potential costs from diseases of barley in Australia. Canberra Australia: Grains Research and Development Corporation.

Murray, T. D. (2008). *Diseases of small grain cereal crops : a colour handbook*. London: Manson.

O'Brien, E. (2005). *Biological control of Pyrenophora teres.* (Master of Science), Letterkenny Institute of Technology, Ireland.

Odvody, G. N., Boosalis, M. G., & Watkins, J. E. (1982). *Development of pseudothecia during progressive saprophytic colonization of wheat straw by Pyrenophora trichostoma*. Paper presented at the Tan Spot of Wheat and Related Diseases Workshop, North Dakota State University Fargo, USA.

Oliver, R., & Osbourn, A. (1995). Molecular dissection of fungal phytopathogenicity. *Microbiology, 141*, 1-9. doi: 10.1099/00221287-141-1-1

Park, R. (2008). Breeding cereals for rust resistance in Australia. *Plant Pathology, 57*(4), 591-602. doi: 10.1111/j.1365-3059.2008.01836.x

Paveley, N., Lockley, D., Vaughan, T., Thomas, J., & Schmidt, K. (2000). Predicting effective fungicide doses through observation of leaf emergence. *Plant Pathology, 49*(6), 748-766. doi: 10.1046/j.1365-3059.2000.00518.x

Peever, T. L., & Milgroom, M. G. (1992). Inheritance of triadimenol resistance in *Pyrenophora teres*. *Phytopathology, 82*, 821-828. doi: 10.1094/Phyto-82-821

Péros, J. P., Nguyen, T. H., Troulet, C., Michel-Romiti, C., & Notteghem, J. L. (2006). Assessment of powdery mildew resistance of grape and *Erysiphe necator* pathogenicity using a laboratory assay. *Vitis, 45*, 29-36.

Pfender, W. F., & Wootke, S. L. (1987). Production of pseudothecia and ascospores by *Pyrenophora tritici-repentis* in response to macronutrient concentrations. *Phytopathology, 77*, 1213-1216. doi: 10.1094/Phyto-77-1213

Platz, G. J., Thorn, S., & Daniel, R. (2007). Consultant's Corner: Spot form net blotch - the pox of the north? *Australian Grain, 17*(2), 1-7.

Powell, W., Machray, G. C., & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science, 1*(7), 215-222. doi: 10.1016/1360-1385(96)86898-1

Ramsay, L., Macaulay, M., Degli Ivanissevich, S., MacLean, K., Cardle, L., Fuller, J., Edwards, K., Tuvesson, S., Morgante, M., & Massari, A. (2000). A simple sequence repeat-based linkage map of barley. *Genetics, 156*(4), 1997-2005.

Rau, D., Attene, G., Brown, A. H., Nanni, L., Maier, F. J., Balmas, V., Saba, E., Schafer, W., & Papa, R. (2007). Phylogeny and evolution of mating-type genes from *Pyrenophora teres*, the causal agent of barley "net blotch" disease. *Curr Genet, 51*(6), 377-392. doi: 10.1007/s00294-007-0126-1

Rau, D., Brown, A. H., Brubaker, C. L., Attene, G., Balmas, V., Saba, E., & Papa, R. (2003). Population genetic structure of *Pyrenophora teres* Drechs. the causal agent of net blotch in Sardinian landraces of barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics, 106*(5), 947-959. doi: 10.1007/s00122-002-1173-0

Ruiz-Roldán, M. C., Maier, F. J., & Schäfer, W. (2001). *PTK1*, a mitogen-activated-protein kinase gene, is required for conidiation, appressorium formation and pathogenicity of *Pyrenophora teres* on barley. *Molecular Plant-Microbe Interactions, 14*, 116-125. doi: 10.1094/MPMI.2001.14.2.116

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). *Diversity Arrays Technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus.* Paper presented at the BioMed Central (BMC) Proceedings

Sarpeleh, A., Tate, M. E., Wallwork, H., Catcheside, D. E., & Able, A. J. (2009). Characterisation of low molecular weight phytotoxins isolated from *Pyrenophora teres*. *Physiological and Molecular Plant Pathology, 73*(6), 154-162. doi: 10.1016/j.pmpp.2009.05.003

Sarpeleh, A., Wallwork, H., Catcheside, D. E., Tate, M. E., & Able, A. J. (2007). Proteinaceous metabolites from *Pyrenophora teres* contribute to symptom development of barley net blotch. *Phytopathology, 97*(8), 907-915. doi: 10.1094/PHYTO-97-8-0907

Schaller, C. W. (1955). Inheritance of resistance to net blotch in barley. *Phytopathology, 45*, 174-176.

Science, C. L. (2006). HRM Assay Design and Analysis CorProtocol™ 6000.

Scott, D. B. (1991). Identity of *Pyrenophora* isolates causing net-type and spot-type lesions on barley. *Mycopathologia, 116*, 29-35. doi: 10.1007/BF00436087

Serenius, M., & Manninen, O. (2006). Prochloraz tolerance of *Pyrenophora teres* population in Finland. *Agricultural and Food Science, 15*(1), 35-42. doi: 10.2137/145960606777245588

Serenius, M., Manninen, O., Wallwork, H., & Williams, K. (2007). Genetic differentiation in *Pyrenophora teres* populations measured with AFLP

markers. *Mycological Research, 111*, 213-223. doi: 10.1016/j.mycres.2006.11.009

Shaikh, S. A. (2012). Measures derived from a 2 x 2 table for an accuracy of a diagnostic test. *Journal of Biometrics & Biostatistics, 2*(5), 1-4. doi: 10.4172/2155-6180.1000128

Sharma, H. S. S. (1984). Assessment of the reaction of some spring barley cultivars to *Pyrenophora teres* using whole plants, detached leaves and toxin bioassay. *Plant Pathology, 34*, 371-376. doi: 10.1111/j.1365-3059.1984.tb01332.x

Shipton, W. A. (1966). Effect of net blotch infection of barley on grain yield and quality. *Australian Journal of Experimental Agriculture and Animal Husbandary, 6*, 437-440. doi: 10.1071/EA9660437

Shipton, W. A., Khan, T. N., & Boyd, W. J. R. (1973). Net blotch of barley. *Review of Plant Pathology, 52*, 269-290.

Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V., & Friesen, T. L. (2014). Evaluation of a *Pyrenophora teres* f. *teres* mapping population reveals multiple independent interactions with a region of barley chromosome 6H. *Fungal Genetics and Biology, 70*, 104-112. doi: 10.1016/j.fgb.2014.07.012

Smedegård-Petersen, V. (1971). *Pyrenophora teres* f. *maculata* f. nov and *Pyrenophora teres* f. *teres* on barley in Denmark *Yearbook of the Royal Veterinary and Agricultural University* (pp. 30). Royal Veterinary and Agricultural University, Copenhagen.

Smedegård-Petersen, V. (1974). Reduction in yield and grain size of barley due to attack by the net blotch fungus *Pyrenophora teres Yearbook of the Royal Veterinary and Agricultural University* (pp. 108-117). Royal Veterinary and Agricultural University, Copenhagen.

Smedegård-Petersen, V. (1977). Isolation of two toxins produced by *Pyrenophora teres* and their significance in disease development of net-spot blotch of barley. *Physiological Plant Pathology, 10*, 203-211. doi: 10.1016/0048-4059(77)90024-8

Statistics, A. B. o. (2005). Agricultural Commodities, Australia, 2003-04. from http://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/7121.0Main+Features12003-04?OpenDocument.

Steffenson, B., Hayes, P., & Kleinhofs, A. (1996). Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. *Theoretical and Applied Genetics, 92*(5), 552-558. doi: 10.1007/BF00224557

Steffenson, B. J., & Webster, R. K. (1992). Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. *Phytopathology, 82*, 170-177. doi: 10.1094/Phyto-82-170

Summerell, B. A., & Burgess, L. W. (1988). Factors influencing production of pseudothecia by *Pyrenophora tritici-repentis Transactions of the British Mycological Society, 90*, 557-562. doi: 10.1016/S0007-1536(88)80059-7

Tekauz, A. (1985). A numerical scale to classify reactions of barley to *Pyrenophora teres*. *Canadian Journal of Plant Pathology, 7*, 181-183. doi: 10.1080/07060668509501499

Tekauz, A. (1990). Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* from Western Canada. *Canadian Journal of Plant Pathology, 12*(2), 141-148. doi: 10.1080/07060669009501017

Turgeon, B. G., & Yoder, O. C. (2000). Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and Biology, 31*(1), 1-5. doi: 10.1006/fgbi.2000.1227

van den Berg, C. G. J., & Rossangel, B. G. (1991). Epidemology of spot-type net blotch on spring barley in Saskatchewan. *Phytopathology*, 1446-1452. doi: 10.1094/Phyto-81-1446

van den Berg, C. G. J., & Rossnagel, B. G. (1990a). Effect of tilt on severity of spot-type net blotch, grain yield and yield components in barley. *Canadian Journal of Plant Science, 70*(2), 473-480. doi: 10.4141/cjps90-055

van den Berg, C. G. J., & Rossnagel, B. G. (1990b). Effects of temperature and leaf wetness period on conidium germination and infection of barley by *Pyrenophora teres*. *Canadian Journal of Plant Pathology, 12*, 263-266. doi: 10.1080/07060669009500997

Vergara, M., Cristani, C., & Vannacci, G. (2003). Differential transcripts in *Pyrenophora graminea* and *Pyrenophora teres* putatively related to pathogenicity. *Journal of Plant Pathology, 85*, 157-164. doi: 10.4454/jpp.v85i3.1026

Wallwork, H. (2007). The role of minimum disease resistance standards for the control of cereal diseases. *Crop and Pasture Science, 58*(6), 588-592. doi: 10.1071/AR06143

Wang, X., Mace, E. S., Platz, G. J., Hunt, C. H., Hickey, L. T., Franckowiak, J. D., & Jordan, D. R. (2015). Spot form of net blotch resistance in barley is under complex genetic control. *Theoretical and Applied Genetics, 128*, 489-499. doi: 10.1007/s00122-014-2447-z

Weiergang, I., Jørgensen, H. J. L., Møller, I. M., Friis, P., & Smedegård-Petersen, V. (2002). Optimization of *in vitro* growth conditions of *Pyrenophora teres* for production of the phytotoxin aspergillomarasmine A. *Physiological amd Molecular Plant Pathology, 60*, 131-140. doi: 10.1006/pmpp.2002.0383

Weiland, J. J., Steffenson, B. J., Cartwright, R. D., & Webster, R. K. (1999). Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. *Phytopathology, 89*, 176-181. doi: 10.1094/PHYTO.1999.89.2.176

Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K. P., Muehlbauer, G. J., Chalmers, K. J., Kleinhofs, A., Huttner, E., & Kilian, A. (2006). A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics, 7*, 206. doi: 10.1186/1471-2164-7-206

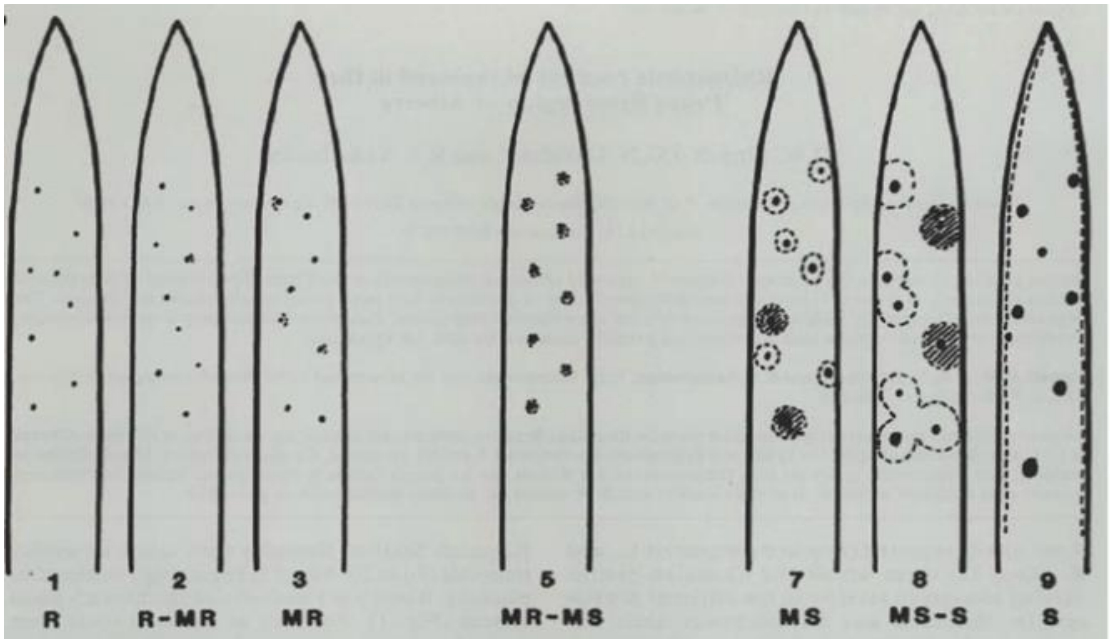
White, H., & Potts, G. (2006). Mutation scanning by high resolution melt analysis. Evaluation of rotor-gene™ 6000 (Corbett Life Science), HR-1™ and 384-well lightscanner™ (Idaho Technology). *National Genetics Reference Laboratory*, 1-45.

Wilcoxson, R. D., Rasmusson, D. C., & Miles, M. R. (1990). Development of barley resistant to spot blotch and genetics of resistance. *Plant Disease, 74*, 207-210. doi: 10.1094/PD-74-0207

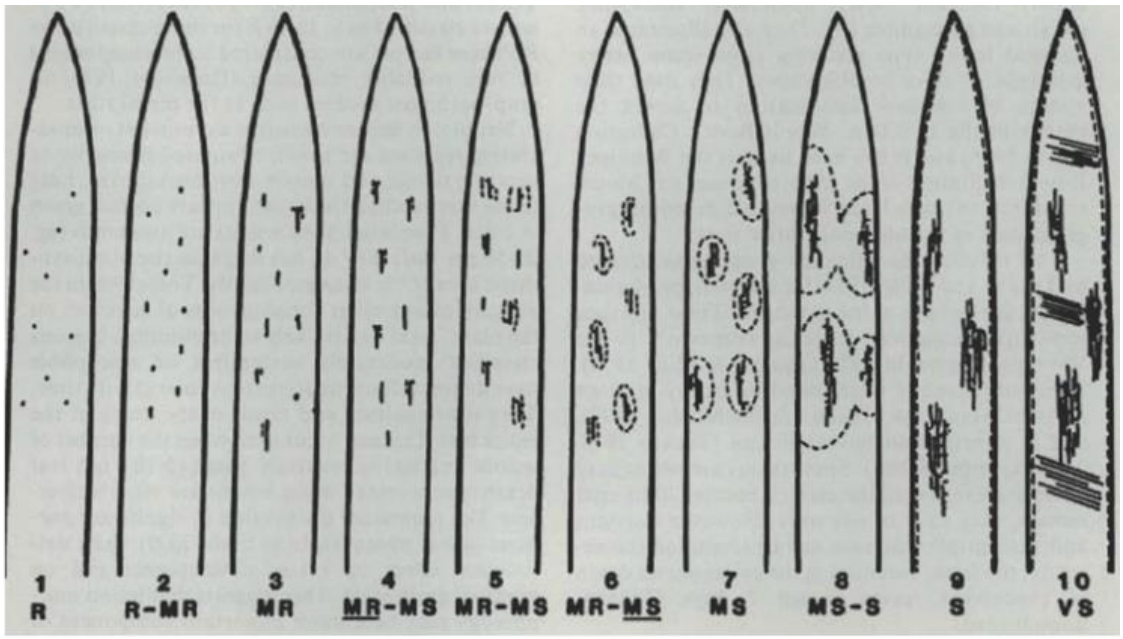
Wilcoxson, R. D., Rasmusson, D. C., Treeful, L. M., & Suganda, T. (1992). Inheritance of resistance to *Pyrenophora teres* in Minnesota barley. *Plant Disease, 76*, 367-369. doi: 10.1094/PD-76-0367

Yoder, O. C. (1983). Use of pathogen-produced toxins in genetic engineering of plants and pathogens. *Genetic Engineering of Plants, 26*, 335-353. doi: 10.1007/978-1-4684-4544-2_23

Appendices:



Appendix 2.1: Tekauz scoring scale SFNB



Appendix 2.2: Tekauz scoring scale

Detached leaf assay scale					
Score	**Lesion size**	**Chlorosis**	**Symptoms**	**Resistance/ Susceptibility**	**Photo**
0	No lesion	No	No symptoms	Highly resistant	
1	Diameter of inoculum drop	No	Very few small brown necrotic lesions	Resistant	
2	Diameter of inoculum drop	No	Small brown necrotic lesions	Resistant	
3	Diameter of inoculum drop	No	Entire inoculum drop looks brown	Resistant	
4	Slightly spreading out of inoculum drop	No or slight	Slight spread of necrotic lesions out of inoculum drop	Moderately resistant	
5	Spreading out of inoculum drop	No or slight	Necrotic lesions spreading further out of inoculum drop	Moderately resistant	
6	Spreading out of inoculum drop	No or slight	Necrotic lesions spreading quite far out of inoculum drop but little or no chlorosis	Moderately resistant	
7	Almost across entire leaf segment	Yes	Brown necrotic lesions with chlorotic regions	Susceptible	
8	Almost across entire leaf segment	Yes	Brown necrotic lesions occupying almost entire leaf surface with chlorotic regions	Susceptible	
9	Almost across entire leaf segment	Yes	Grey brown necrotic lesion with chlorosis; mycelium growth visible	Susceptible	

Appendix 2.3: The scale used for scoring DLA – droplet method

Appendix 3.1 Scores for population 9 (NB029 x NB085). The green coloured cells indicate resistant reactions; the red cells indicate susceptible reactions while the yellow coloured cells indicate intermediate reactions.

Isolate	Genotype	Iso	var	serial	Day 14	Comments
NB85	CI11458	89	1	1	1.50	
NB85	Fleet	89	3	1	1.50	
NB85	Corvette	89	9	1	3.67	
NB85	Harbin	89	7	1	1.00	
NB85	Vlamingh	89	15	1	1.00	
NB85	Algerian	89	2	1	1.00	
NB85	Kombar	89	10	1	1.67	
NB85	Buloke	89	13	1	1.50	
NB85	Yerong	89	5	1	1.00	
NB85	Gilbert	89	12	1	1.00	
NB85	Beecher	89	4	1	1.00	
NB85	Rojo	89	14	1	1.00	
NB85	Maritime	89	6	1	3.33	
NB85	Prior	89	8	1	1.00	
NB85	Skiff	89	11	1	1.00	
Isolate04	Buloke	4	13	2	1.00	
Isolate04	Harbin	4	7	2	1.00	
Isolate04	Rojo	4	14	2	1.50	
Isolate04	Yerong	4	5	2	3.00	
Isolate04	Gilbert	4	12	2	6.00	
Isolate04	Skiff	4	11	2	1.00	
Isolate04	Corvette	4	9	2	5.67	
Isolate04	Fleet	4	3	2	3.33	
Isolate04	CI11458	4	1	2	1.67	
Isolate04	Kombar	4	10	2	4.33	
Isolate04	Maritime	4	6	2	3.00	
Isolate04	Vlamingh	4	15	2	1.00	
Isolate04	Beecher	4	4	2	6.00	
Isolate04	Algerian	4	2	2	2.00	
Isolate22	CI11458	22	1	4	3.50	
Isolate22	Algerian	22	2	4	4.50	
Isolate22	Maritime	22	6	4	2.50	
Isolate22	Gilbert	22	12	4	4.00	
Isolate22	Vlamingh	22	15	4	1.00	
Isolate22	Prior	22	8	4	2.00	
Isolate22	Yerong	22	5	4	2.00	
Isolate22	Beecher	22	4	4	2.00	
Isolate22	Fleet	22	3	4	3.50	
Isolate22	Rojo	22	14	4	1.00	
Isolate22	Corvette	22	9	4	4.00	
Isolate22	Kombar	22	10	4	1.50	
Isolate22	Harbin	22	7	4	1.00	
Isolate22	Skiff	22	11	4	2.00	
Isolate22	Buloke	22	13	4	3.33	
Isolate54	Skiff	54	11	6	1.00	
Isolate54	Prior	54	8	6	5.00	
Isolate54	Corvette	54	9	6	7.00	
Isolate54	Rojo	54	14	6	1.00	
Isolate54	Vlamingh	54	15	6	1.00	
Isolate54	Gilbert	54	12	6	5.00	
Isolate54	Fleet	54	3	6	5.50	

Isolate54	CI11458	54	1	6	6.00
Isolate54	Harbin	54	7	6	4.00
Isolate54	Algerian	54	2	6	6.00
Isolate54	Maritime	54	6	6	5.00
Isolate54	Yerong	54	5	6	1.00
Isolate54	Beecher	54	4	6	5.00
Isolate54	Buloke	54	13	6	2.00
Isolate54	Kombar	54	10	6	3.00
NB29	Gilbert	88	12	7	3.00
NB29	Buloke	88	13	7	4.00
NB29	Algerian	88	2	7	1.00
NB29	Skiff	88	11	7	1.00
NB29	Harbin	88	7	7	1.00
NB29	Maritime	88	6	7	3.00
NB29	Corvette	88	9	7	3.00
NB29	Rojo	88	14	7	1.00
NB29	Kombar	88	10	7	1.00
NB29	Beecher	88	4	7	5.50
NB29	Yerong	88	5	7	1.50
NB29	Prior	88	8	7	2.00
NB29	Vlamingh	88	15	7	3.00
NB29	CI11458	88	1	7	2.00
NB29	Fleet	88	3	7	1.00
Isolate68	Kombar	68	10	8	1.00
Isolate68	Rojo	68	14	8	1.00
Isolate68	Fleet	68	3	8	4.50
Isolate68	Algerian	68	2	8	1.00
Isolate68	Prior	68	8	8	3.00
Isolate68	Beecher	68	4	8	4.00
Isolate68	Maritime	68	6	8	6.67
Isolate68	Yerong	68	5	8	2.00
Isolate68	Vlamingh	68	15	8	1.00
Isolate68	CI11458	68	1	8	3.00
Isolate68	Buloke	68	13	8	4.00
Isolate68	Harbin	68	7	8	1.00
Isolate68	Skiff	68	11	8	1.50
Isolate68	Corvette	68	9	8	6.33
Isolate68	Gilbert	68	12	8	3.00
Isolate28	Maritime	28	6	9	3.50
Isolate28	Kombar	28	10	9	3.50
Isolate28	Gilbert	28	12	9	4.00
Isolate28	Buloke	28	13	9	5.00
Isolate28	Beecher	28	4	9	3.00
Isolate28	CI11458	28	1	9	3.50
Isolate28	Rojo	28	14	9	1.50
Isolate28	Prior	28	8	9	5.00
Isolate28	Vlamingh	28	15	9	1.00
Isolate28	Harbin	28	7	9	3.00
Isolate28	Algerian	28	2	9	3.00
Isolate28	Skiff	28	11	9	3.00
Isolate28	Fleet	28	3	9	5.00
Isolate28	Corvette	28	9	9	7.00
Isolate28	Yerong	28	5	9	1.00
Isolate11	Prior	11	8	10	3.00
Isolate11	Algerian	11	2	10	2.00
Isolate11	Skiff	11	11	10	1.00

Isolate11	Maritime	11	6	10	2.00
Isolate11	Corvette	11	9	10	6.33
Isolate11	Fleet	11	3	10	1.50
Isolate11	Gilbert	11	12	10	2.00
Isolate11	Harbin	11	7	10	2.33
Isolate11	Beecher	11	4	10	1.50
Isolate11	Vlamingh	11	15	10	1.00
Isolate11	Yerong	11	5	10	2.00
Isolate11	Buloke	11	13	10	1.00
Isolate11	CI11458	11	1	10	5.00
Isolate11	Kombar	11	10	10	1.50
Isolate11	Rojo	11	14	10	1.00
Isolate36	Prior	36	8	11	3.50
Isolate36	Rojo	36	14	11	1.00
Isolate36	Harbin	36	7	11	1.00
Isolate36	Yerong	36	5	11	2.00
Isolate36	Skiff	36	11	11	1.00
Isolate36	Kombar	36	10	11	2.00
Isolate36	Vlamingh	36	15	11	2.00
Isolate36	Corvette	36	9	11	6.00
Isolate36	CI11458	36	1	11	4.00
Isolate36	Gilbert	36	12	11	5.67
Isolate36	Buloke	36	13	11	4.67
Isolate36	Algerian	36	2	11	3.00
Isolate36	Fleet	36	3	11	5.00
Isolate36	Maritime	36	6	11	5.33
Isolate36	Beecher	36	4	11	3.33
Isolate55	Kombar	55	10	12	2.00
Isolate55	Skiff	55	11	12	1.00
Isolate55	Vlamingh	55	15	12	2.50
Isolate55	Harbin	55	7	12	2.67
Isolate55	Beecher	55	4	12	5.50
Isolate55	Corvette	55	9	12	5.00
Isolate55	Buloke	55	13	12	1.50
Isolate55	Gilbert	55	12	12	3.00
Isolate55	Maritime	55	6	12	2.67
Isolate55	Fleet	55	3	12	4.67
Isolate55	CI11458	55	1	12	1.50
Isolate55	Prior	55	8	12	5.50
Isolate55	Yerong	55	5	12	2.00
Isolate55	Rojo	55	14	12	2.50
Isolate55	Algerian	55	2	12	1.00
Isolate35	Harbin	35	7	13	2.00
Isolate35	Kombar	35	10	13	4.67
Isolate35	Vlamingh	35	15	13	2.50
Isolate35	Corvette	35	9	13	5.50
Isolate35	Prior	35	8	13	4.00
Isolate35	Buloke	35	13	13	1.50
Isolate35	Beecher	35	4	13	4.00
Isolate35	Yerong	35	5	13	2.00
Isolate35	Maritime	35	6	13	1.50
Isolate35	Rojo	35	14	13	1.50
Isolate35	CI11458	35	1	13	2.33
Isolate35	Skiff	35	11	13	1.00
Isolate35	Algerian	35	2	13	1.00
Isolate35	Gilbert	35	12	13	3.50

very consistent

Isolate35	Fleet	35	3	13	3.67
Isolate30	Buloke	30	13	14	4.50
Isolate30	Fleet	30	3	14	1.00
Isolate30	Algerian	30	2	14	1.00
Isolate30	Beecher	30	4	14	1.00
Isolate30	Maritime	30	6	14	2.50
Isolate30	Yerong	30	5	14	1.00
Isolate30	Kombar	30	10	14	1.00
Isolate30	Rojo	30	14	14	1.00
Isolate30	Vlamingh	30	15	14	1.00
Isolate30	Corvette	30	9	14	3.00
Isolate30	Gilbert	30	12	14	3.50
Isolate30	Harbin	30	7	14	1.00
Isolate30	Skiff	30	11	14	2.00
Isolate30	Prior	30	8	14	2.50
Isolate30	CI11458	30	1	14	2.00
Isolate13	CI11458	13	1	15	2.00
Isolate13	Maritime	13	6	15	3.00
Isolate13	Corvette	13	9	15	3.00
Isolate13	Buloke	13	13	15	2.00
Isolate13	Vlamingh	13	15	15	1.00
Isolate13	Rojo	13	14	15	1.50
Isolate13	Skiff	13	11	15	1.00
Isolate13	Fleet	13	3	15	2.00
Isolate13	Beecher	13	4	15	2.00
Isolate13	Kombar	13	10	15	1.00
Isolate13	Algerian	13	2	15	2.00
Isolate13	Gilbert	13	12	15	1.50
Isolate13	Yerong	13	5	15	1.00
Isolate13	Harbin	13	7	15	1.00
Isolate13	Prior	13	8	15	1.00
Isolate41	Beecher	41	4	16	7.00
Isolate41	Vlamingh	41	15	16	1.00
Isolate41	Skiff	41	11	16	2.00
Isolate41	CI11458	41	1	16	3.00
Isolate41	Corvette	41	9	16	6.00
Isolate41	Yerong	41	5	16	2.00
Isolate41	Prior	41	8	16	3.50
Isolate41	Harbin	41	7	16	1.67
Isolate41	Gilbert	41	12	16	3.67
Isolate41	Rojo	41	14	16	2.00
Isolate41	Maritime	41	6	16	6.33
Isolate41	Fleet	41	3	16	4.00
Isolate41	Algerian	41	2	16	2.50
Isolate41	Buloke	41	13	16	3.00
Isolate41	Kombar	41	10	16	3.67
Isolate07	CI11458	7	1	17	4.50
Isolate07	Fleet	7	3	17	2.67
Isolate07	Corvette	7	9	17	4.00
Isolate07	Harbin	7	7	17	2.50
Isolate07	Vlamingh	7	15	17	2.00
Isolate07	Algerian	7	2	17	2.50
Isolate07	Kombar	7	10	17	3.00
Isolate07	Buloke	7	13	17	2.50
Isolate07	Yerong	7	5	17	2.50
Isolate07	Gilbert	7	12	17	3.33

Isolate07	Beecher	7	4	17	4.00	
Isolate07	Rojo	7	14	17	2.33	
Isolate07	Maritime	7	6	17	3.33	
Isolate07	Prior	7	8	17	3.33	
Isolate07	Skiff	7	11	17	3.00	
Isolate09	Buloke	9	13	18	4.67	
Isolate09	Harbin	9	7	18	1.67	
Isolate09	Rojo	9	14	18	3.50	
Isolate09	Yerong	9	5	18	1.00	1st leaf, 6
Isolate09	Gilbert	9	12	18	4.00	
Isolate09	Skiff	9	11	18	1.00	
Isolate09	Corvette	9	9	18	6.33	
Isolate09	Fleet	9	3	18	5.00	
Isolate09	CI11458	9	1	18	2.00	1st leaf, 6
Isolate09	Prior	9	8	18	4.67	
Isolate09	Kombar	9	10	18	1.00	1st leaf, 4
Isolate09	Maritime	9	6	18	3.00	
Isolate09	Vlamingh	9	15	18	1.00	
Isolate09	Beecher	9	4	18	4.33	
Isolate09	Algerian	9	2	18	2.50	
Isolate21	Kombar	21	10	1	6.00	
Isolate21	Harbin	21	7	1	5.00	
Isolate21	CI11458	21	1	1	2.00	
Isolate21	Corvette	21	9	1	7.50	
Isolate21	Buloke	21	13	1	3.50	
Isolate21	Fleet	21	3	1	5.50	
Isolate21	Yerong	21	5	1	6.00	welting
Isolate21	Gilbert	21	12	1	4.00	

Isolate21	Algerian	21	2	1	5.00	
Isolate21	Beecher	21	4	1	8.00	
Isolate21	Skiff	21	11	1	4.50	
Isolate21	Prior	21	8	1	7.50	
Isolate21	Rojo	21	14	1	4.00	
Isolate21	Maritime	21	6	1	7.00	
Isolate21	Vlamingh	21	15	1	3.00	
Isolate34	Skiff	34	11	2	2.50	
Isolate34	Corvette	34	9	2	4.50	
Isolate34	Buloke	34	13	2	2.00	
Isolate34	Algerian	34	2	2	5.00	
Isolate34	Yerong	34	5	2	7.00	
Isolate34	Rojo	34	14	2	2.00	
Isolate34	Beecher	34	4	2	6.00	
Isolate34	Maritime	34	6	2	7.00	
Isolate34	Fleet	34	3	2	4.50	
Isolate34	CI11458	34	1	2	2.00	
Isolate34	Vlamingh	34	15	2	2.00	
Isolate34	Harbin	34	7	2	2.00	1st leaf, 6
Isolate34	Gilbert	34	12	2	3.00	welting
Isolate34	Prior	34	8	2	3.00	
Isolate34	Kombar	34	10	2	3.50	1st leaf, 7
NB85	Gilbert	89	12	3	7.50	
NB85	Rojo	89	14	3	2.00	
NB85	Skiff	89	11	3	4.00	
NB85	Algerian	89	2	3	7.00	
NB85	Prior	89	8	3	7.00	
NB85	Beecher	89	4	3	4.00	

NB85	Buloke	89	13	3	5.50	1st leaf, 7
NB85	Yerong	89	5	3	2.00	
NB85	Kombar	89	10	3	2.00	
NB85	Maritime	89	6	3	4.50	
NB85	Fleet	89	3	3	5.50	
NB85	Harbin	89	7	3	5.50	
NB85	Vlamingh	89	15	3	2.00	
NB85	CI11458	89	1	3	2.00	
NB85	Corvette	89	9	3	8.00	
Isolate62	Yerong	62	5	5	2.00	
Isolate62	Harbin	62	7	5	6.00	
Isolate62	Rojo	62	14	5	2.00	
Isolate62	Prior	62	8	5	2.00	
Isolate62	Kombar	62	10	5	5.50	
Isolate62	Gilbert	62	12	5	2.00	
Isolate62	Skiff	62	11	5	2.50	
Isolate62	Beecher	62	4	5	2.00	
Isolate62	Algerian	62	2	5	2.00	
Isolate62	Fleet	62	3	5	2.00	
Isolate62	Vlamingh	62	15	5	3.00	
Isolate62	Maritime	62	6	5	4.50	
Isolate62	Corvette	62	9	5	4.00	
Isolate62	Buloke	62	13	5	2.00	
Isolate62	CI11458	62	1	5	2.00	
Isolate44	Vlamingh	44	15	6	4.00	
Isolate44	Kombar	44	10	6	2.00	1st leaf, 5
Isolate44	Algerian	44	2	6	2.00	
Isolate44	Beecher	44	4	6	2.00	

Isolate44	Rojo	44	14	6	2.00	welting
Isolate44	Fleet	44	3	6	2.00	
Isolate44	Prior	44	8	6	8.00	
Isolate44	Maritime	44	6	6	4.00	
Isolate44	Corvette	44	9	6	2.00	
Isolate44	Buloke	44	13	6	3.50	
Isolate44	CI11458	44	1	6	3.50	
Isolate44	Gilbert	44	12	6	2.00	
Isolate44	Yerong	44	5	6	3.50	
Isolate44	Harbin	44	7	6	3.50	
Isolate44	Skiff	44	11	6	3.50	
Isolate40	Skiff	40	11	7	5.00	
Isolate40	Vlamingh	40	15	7	2.00	
Isolate40	Gilbert	40	12	7	6.50	
Isolate40	CI11458	40	1	7	5.50	
Isolate40	Yerong	40	5	7	4.50	
Isolate40	Buloke	40	13	7	4.00	
Isolate40	Algerian	40	2	7	4.00	
Isolate40	Corvette	40	9	7	7.00	
Isolate40	Harbin	40	7	7	6.50	
Isolate40	Prior	40	8	7	4.50	
Isolate40	Fleet	40	3	7	4.50	
Isolate40	Rojo	40	14	7	5.00	
Isolate40	Maritime	40	6	7	4.00	
Isolate40	Kombar	40	10	7	3.00	
Isolate40	Beecher	40	4	7	3.50	
Isolate16	CI11458	16	1	8	7.00	
Isolate16	Rojo	16	14	8	4.00	

Isolate16	Corvette	16	9	8	7.00	
Isolate16	Maritime	16	6	8	8.00	
Isolate16	Prior	16	8	8	7.50	
Isolate16	Harbin	16	7	8	4.00	
Isolate16	Yerong	16	5	8	8.00	
Isolate16	Beecher	16	4	8	7.50	
Isolate16	Vlamingh	16	15	8	3.00	
Isolate16	Kombar	16	10	8	2.50	
Isolate16	Buloke	16	13	8	4.50	
Isolate16	Gilbert	16	12	8	2.50	
Isolate16	Skiff	16	11	8	2.00	
Isolate16	Algerian	16	2	8	#DIV/0!	no growth
Isolate16	Fleet	16	3	8	2.00	
NB29	Prior	88	8	9	6.50	
NB29	Rojo	88	14	9	2.00	
NB29	Gilbert	88	12	9	4.50	
NB29	Beecher	88	4	9	8.00	
NB29	Maritime	88	6	9	7.00	
NB29	CI11458	88	1	9	4.50	
NB29	Vlamingh	88	15	9	4.00	
NB29	Buloke	88	13	9	3.00	
NB29	Skiff	88	11	9	4.00	
NB29	Corvette	88	9	9	6.50	
NB29	Kombar	88	10	9	8.50	
NB29	Fleet	88	3	9	4.00	
NB29	Harbin	88	7	9	3.50	
NB29	Algerian	88	2	9	6.00	
NB29	Yerong	88	5	9	6.00	

Isolate30	Fleet	30	3	10	3.50	
Isolate30	Yerong	30	5	10	5.50	
Isolate30	Beecher	30	4	10	4.00	
Isolate30	Harbin	30	7	10	3.00	
Isolate30	Gilbert	30	12	10	6.00	
Isolate30	Vlamingh	30	15	10	3.00	
Isolate30	Prior	30	8	10	10.00	totally dry
Isolate30	Rojo	30	14	10	5.00	
Isolate30	Kombar	30	10	10	8.00	
Isolate30	Buloke	30	13	10	9.00	
Isolate30	Maritime	30	6	10	2.50	
Isolate30	Algerian	30	2	10	8.00	
Isolate30	Skiff	30	11	10	5.00	
Isolate30	Corvette	30	9	10	6.50	
Isolate30	CI11458	30	1	10	4.00	
Isolate14	Vlamingh	14	15	11	3.50	
Isolate14	Yerong	14	5	11	4.00	
Isolate14	Prior	14	8	11	6.00	
Isolate14	Buloke	14	13	11	3.00	
Isolate14	CI11458	14	1	11	3.00	
Isolate14	Maritime	14	6	11	4.50	
Isolate14	Fleet	14	3	11	4.50	
Isolate14	Beecher	14	4	11	6.50	
Isolate14	Harbin	14	7	11	3.00	
Isolate14	Algerian	14	2	11	2.00	
Isolate14	Gilbert	14	12	11	3.50	
Isolate14	Corvette	14	9	11	4.00	
Isolate14	Kombar	14	10	11	3.00	

Isolate14	Rojo	14	14	11	2.00
Isolate14	Skiff	14	11	11	3.00
Isolate64	Algerian	64	2	12	7.00
Isolate64	Fleet	64	3	12	6.00
Isolate64	Kombar	64	10	12	6.00
Isolate64	Rojo	64	14	12	4.00
Isolate64	Vlamingh	64	15	12	3.00
Isolate64	Skiff	64	11	12	6.00
Isolate64	Corvette	64	9	12	8.00
Isolate64	Gilbert	64	12	12	5.50
Isolate64	Maritime	64	6	12	7.00
Isolate64	Beecher	64	4	12	6.00
Isolate64	CI11458	64	1	12	4.50
Isolate64	Yerong	64	5	12	2.00
Isolate64	Prior	64	8	12	5.00
Isolate64	Harbin	64	7	12	2.50
Isolate64	Buloke	64	13	12	2.00
Isolate28	Buloke	28	13	13	3.50
Isolate28	Beecher	28	4	13	3.50
Isolate28	Corvette	28	9	13	6.50
Isolate28	Yerong	28	5	13	8.00
Isolate28	Skiff	28	11	13	5.50
Isolate28	Maritime	28	6	13	2.00
Isolate28	Fleet	28	3	13	6.00
Isolate28	CI11458	28	1	13	2.50
Isolate28	Vlamingh	28	15	13	2.00
Isolate28	Rojo	28	14	13	2.00
Isolate28	Harbin	28	7	13	4.50

Isolate28	Algerian	28	2	13	3.50
Isolate28	Gilbert	28	12	13	3.50
Isolate28	Prior	28	8	13	3.00
Isolate28	Kombar	28	10	13	2.00
Isolate08	Maritime	8	6	14	2.00
Isolate08	Skiff	8	11	14	2.00
Isolate08	Fleet	8	3	14	2.00
Isolate08	Buloke	8	13	14	2.00
Isolate08	Algerian	8	2	14	2.00
Isolate08	CI11458	8	1	14	2.00
Isolate08	Gilbert	8	12	14	2.00
Isolate08	Kombar	8	10	14	2.00
Isolate08	Harbin	8	7	14	2.00
Isolate08	Yerong	8	5	14	2.00
Isolate08	Corvette	8	9	14	2.00
Isolate08	Prior	8	8	14	2.00
Isolate08	Vlamingh	8	15	14	2.00
Isolate08	Beecher	8	4	14	2.00
Isolate08	Rojo	8	14	14	2.00
Isolate38	Prior	38	8	15	5.00
Isolate38	Fleet	38	3	15	5.50
Isolate38	Buloke	38	13	15	2.00
Isolate38	Vlamingh	38	15	15	3.50
Isolate38	Kombar	38	10	15	2.00
Isolate38	Skiff	38	11	15	2.00
Isolate38	CI11458	38	1	15	7.00
Isolate38	Rojo	38	14	15	3.00
Isolate38	Gilbert	38	12	15	4.00

1st leaf, 6-7

Isolate38	Maritime	38	6	15	8.00
Isolate38	Algerian	38	2	15	6.00
Isolate38	Beecher	38	4	15	5.50
Isolate38	Harbin	38	7	15	2.50
Isolate38	Corvette	38	9	15	2.50
Isolate38	Yerong	38	5	15	5.00
Isolate05	Harbin	5	7	16	3.00
Isolate05	Algerian	5	2	16	2.00
Isolate05	Yerong	5	5	16	2.00
Isolate05	Gilbert	5	12	16	4.00
Isolate05	Fleet	5	3	16	5.00
Isolate05	Beecher	5	4	16	3.00
Isolate05	Maritime	5	6	16	5.50
Isolate05	Prior	5	8	16	6.50
Isolate05	Kombar	5	10	16	3.00
Isolate05	Corvette	5	9	16	3.00
Isolate05	Skiff	5	11	16	3.00
Isolate05	CI11458	5	1	16	3.00
Isolate05	Rojo	5	14	16	2.50
Isolate05	Buloke	5	13	16	2.50
Isolate05	Vlamingh	5	15	16	2.50
Isolate23	Kombar	23	10	17	4.50
Isolate23	Harbin	23	7	17	2.00
Isolate23	CI11458	23	1	17	3.00
Isolate23	Corvette	23	9	17	6.00
Isolate23	Buloke	23	13	17	5.00
Isolate23	Fleet	23	3	17	2.00
Isolate23	Yerong	23	5	17	2.00
Isolate23	Gilbert	23	12	17	4.50
Isolate23	Algerian	23	2	17	4.00
Isolate23	Beecher	23	4	17	7.00
Isolate23	Skiff	23	11	17	5.50
Isolate23	Prior	23	8	17	4.50
Isolate23	Rojo	23	14	17	3.00
Isolate23	Maritime	23	6	17	5.00
Isolate23	Vlamingh	23	15	17	3.00
Isolate65	Skiff	65	11	18	2.00
Isolate65	Corvette	65	9	18	2.00
Isolate65	Buloke	65	13	18	2.00
Isolate65	Algerian	65	2	18	2.00
Isolate65	Yerong	65	5	18	2.00
Isolate65	Rojo	65	14	18	2.00
Isolate65	Beecher	65	4	18	2.00
Isolate65	Maritime	65	6	18	2.00
Isolate65	Fleet	65	3	18	3.00
Isolate65	CI11458	65	1	18	2.00
Isolate65	Vlamingh	65	15	18	2.00
Isolate65	Harbin	65	7	18	2.00
Isolate65	Gilbert	65	12	18	2.00
Isolate65	Prior	65	8	18	2.00
Isolate65	Kombar	65	10	18	2.00
NB29	Maritime	88	6	2	9.50
NB29	Harbin	88	7	2	4.50
NB29	Rojo	88	14	2	3.50
NB29	Vlamingh	88	15	2	2.50
NB29	Corvette	88	9	2	9.50

NB29	Algerian	88	2	2	4.50		control	Harbin	54	7	4	0.00	
NB29	Fleet	88	3	2	7.00	Fungal growth	control	CI11458	54	1	4	0.00	
NB29	Buloke	88	13	2	3.00		control	Buloke	54	13	4	0.00	
NB29	Prior	88	8	2	5.50		control	Algerian	54	2	4	0.00	
NB29	CI11458	88	1	2	4.50		control	Yerong	54	5	4	0.00	
NB29	Skiff	88	11	2	4.00		control	Maritime	54	6	4	0.00	
NB29	Kombar	88	10	2	0.00	, 2nd leaf barel germinated_1st leaf, 6-7	control	Gilbert	54	12	4	0.00	
NB29	Beecher	88	4	2	7.50		control	Beecher	54	4	4	0.00	
NB29	Gilbert	88	12	2	4.00		control	Fleet	54	3	4	0.00	
NB29	Yerong	88	5	2	2.00	1st leaf, 4	control	Kombar	54	10	4	0.00	
Isolate70	Algerian	70	2	3	3.50		control	Rojo	54	14	4	0.00	
Isolate70	Buloke	70	13	3	3.50		control	Skiff	54	11	4	0.00	
Isolate70	Rojo	70	14	3	2.00		NB85	Rojo	89	14	5	3.00	
Isolate70	Beecher	70	4	3	2.50		NB85	Vlamingh	89	15	5	3.00	
Isolate70	Fleet	70	3	3	2.33		NB85	Buloke	89	13	5	4.00	
Isolate70	Skiff	70	11	3	2.50		NB85	Corvette	89	9	5	7.00	Fungal growth
Isolate70	Gilbert	70	12	3	3.00		NB85	Kombar	89	10	5	3.50	
Isolate70	Kombar	70	10	3	3.00		NB85	Skiff	89	11	5	5.50	
Isolate70	CI11458	70	1	3	2.50		NB85	Beecher	89	4	5	4.00	
Isolate70	Prior	70	8	3	5.00		NB85	Harbin	89	7	5	4.67	
Isolate70	Yerong	70	5	3	3.00		NB85	Prior	89	8	5	7.00	Fungal growth
Isolate70	Vlamingh	70	15	3	2.00		NB85	Yerong	89	5	5	6.00	
Isolate70	Maritime	70	6	3	2.00		NB85	Algerian	89	2	5	6.50	
Isolate70	Corvette	70	9	3	5.00		NB85	Maritime	89	6	5	4.50	
Isolate70	Harbin	70	7	3	1.00		NB85	CI11458	89	1	5	3.00	
control	Prior	54	8	4	0.00		NB85	Gilbert	89	12	5	4.50	
control	Vlamingh	54	15	4	0.00		NB85	Fleet	89	3	5	5.50	
control	Corvette	54	9	4	0.00		Isolate38	Harbin	38	7	6	3.50	

Isolate38	Kombar	38	10	6	5.50
Isolate38	Yerong	38	5	6	4.00
Isolate38	Vlamingh	38	15	6	2.50
Isolate38	Maritime	38	6	6	6.00
Isolate38	Fleet	38	3	6	6.00
Isolate38	Algerian	38	2	6	6.00
Isolate38	Gilbert	38	12	6	5.50
Isolate38	Skiff	38	11	6	4.00
Isolate38	Beecher	38	4	6	7.50
Isolate38	Buloke	38	13	6	3.00
Isolate38	CI11458	38	1	6	4.67
Isolate38	Rojo	38	14	6	4.00
Isolate38	Prior	38	8	6	9.50
Isolate38	Corvette	38	9	6	5.50
Isolate22	Vlamingh	22	15	7	2.50
Isolate22	Gilbert	22	12	7	4.50
Isolate22	Harbin	22	7	7	3.00
Isolate22	Kombar	22	10	7	3.00
Isolate22	Prior	22	8	7	10.00
Isolate22	Fleet	22	3	7	5.50
Isolate22	Rojo	22	14	7	3.50
Isolate22	Yerong	22	5	7	8.00
Isolate22	Beecher	22	4	7	3.50
Isolate22	Buloke	22	13	7	3.50
Isolate22	CI11458	22	1	7	4.00
Isolate22	Algerian	22	2	7	5.50
Isolate22	Maritime	22	6	7	4.50
Isolate22	Skiff	22	11	7	4.50

1st leaf, 10

Isolate22	Corvette	22	9	7	9.00
Isolate47	Maritime	47	6	8	4.50
Isolate47	Yerong	47	5	8	3.50
Isolate47	Buloke	47	13	8	2.50
Isolate47	CI11458	47	1	8	2.00
Isolate47	Algerian	47	2	8	3.00
Isolate47	Rojo	47	14	8	1.00
Isolate47	Corvette	47	9	8	6.00
Isolate47	Fleet	47	3	8	4.50
Isolate47	Harbin	47	7	8	2.50
Isolate47	Gilbert	47	12	8	3.50
Isolate47	Skiff	47	11	8	1.50
Isolate47	Prior	47	8	8	4.00
Isolate47	Kombar	47	10	8	5.00
Isolate47	Vlamingh	47	15	8	1.00
Isolate47	Beecher	47	4	8	3.00
Isolate41	Yerong	41	5	9	2.50
Isolate41	Corvette	41	9	9	6.50
Isolate41	Gilbert	41	12	9	3.00
Isolate41	Skiff	41	11	9	4.50
Isolate41	Buloke	41	13	9	4.00
Isolate41	CI11458	41	1	9	3.50
Isolate41	Algerian	41	2	9	4.00
Isolate41	Beecher	41	4	9	4.50
Isolate41	Kombar	41	10	9	4.00
Isolate41	Prior	41	8	9	7.00
Isolate41	Harbin	41	7	9	3.00
Isolate41	Maritime	41	6	9	7.00

Fungal growth

Fungal growth

Isolate41	Fleet	41	3	9	5.00
Isolate41	Rojo	41	14	9	1.00
Isolate41	Vlamingh	41	15	9	2.50
Isolate45	Buloke	45	13	10	1.00
Isolate45	Beecher	45	4	10	5.00
Isolate45	Kombar	45	10	10	2.50
Isolate45	Harbin	45	7	10	1.00
Isolate45	Maritime	45	6	10	5.50
Isolate45	Yerong	45	5	10	3.00
Isolate45	Vlamingh	45	15	10	2.00
Isolate45	CI11458	45	1	10	3.00
Isolate45	Corvette	45	9	10	5.50
Isolate45	Fleet	45	3	10	4.00
Isolate45	Rojo	45	14	10	3.00
Isolate45	Gilbert	45	12	10	5.00
Isolate45	Skiff	45	11	10	2.50
Isolate45	Prior	45	8	10	7.00
Isolate45	Algerian	45	2	10	3.00
Isolate55	CI11458	55	1	11	1.50
Isolate55	Maritime	55	6	11	5.00
Isolate55	Yerong	55	5	11	4.00
Isolate55	Prior	55	8	11	5.00
Isolate55	Algerian	55	2	11	2.00
Isolate55	Kombar	55	10	11	3.00
Isolate55	Harbin	55	7	11	4.00
Isolate55	Fleet	55	3	11	4.00
Isolate55	Rojo	55	14	11	2.00
Isolate55	Skiff	55	11	11	3.00

Fungal growth

Isolate55	Corvette	55	9	11	6.00
Isolate55	Buloke	55	13	11	3.00
Isolate55	Gilbert	55	12	11	4.00
Isolate55	Beecher	55	4	11	5.00
Isolate55	Vlamingh	55	15	11	2.00
Isolate07	Kombar	7	10	12	7.00
Isolate07	Harbin	7	7	12	3.50
Isolate07	Gilbert	7	12	12	7.00
Isolate07	Yerong	7	5	12	8.50
Isolate07	Corvette	7	9	12	7.50
Isolate07	Vlamingh	7	15	12	2.00
Isolate07	Prior	7	8	12	7.00
Isolate07	Skiff	7	11	12	4.50
Isolate07	Beecher	7	4	12	5.50
Isolate07	CI11458	7	1	12	6.00
Isolate07	Rojo	7	14	12	3.50
Isolate07	Maritime	7	6	12	7.50
Isolate07	Fleet	7	3	12	5.00
Isolate07	Algerian	7	2	12	6.50
Isolate07	Buloke	7	13	12	3.00
Isolate35	Skiff	35	11	13	1.00
Isolate35	Beecher	35	4	13	4.00
Isolate35	Maritime	35	6	13	3.00
Isolate35	Gilbert	35	12	13	2.33
Isolate35	CI11458	35	1	13	2.50
Isolate35	Rojo	35	14	13	1.00
Isolate35	Yerong	35	5	13	4.50
Isolate35	Fleet	35	3	13	1.00

Isolate35	Kombar	35	10	13	5.00
Isolate35	Vlamingh	35	15	13	1.00
Isolate35	Prior	35	8	13	3.00
Isolate35	Corvette	35	9	13	5.50
Isolate35	Buloke	35	13	13	1.00
Isolate35	Harbin	35	7	13	4.33
Isolate35	Algerian	35	2	13	2.67
Isolate44	Corvette	44	9	14	5.00
Isolate44	Prior	44	8	14	6.50
Isolate44	CI11458	44	1	14	6.67
Isolate44	Yerong	44	5	14	7.50
Isolate44	Algerian	44	2	14	6.50
Isolate44	Harbin	44	7	14	3.50
Isolate44	Buloke	44	13	14	4.00
Isolate44	Maritime	44	6	14	4.50
Isolate44	Vlamingh	44	15	14	2.50
Isolate44	Gilbert	44	12	14	4.33
Isolate44	Rojo	44	14	14	3.00
Isolate44	Fleet	44	3	14	8.33
Isolate44	Beecher	44	4	14	2.50
Isolate44	Skiff	44	11	14	3.00
Isolate44	Kombar	44	10	14	4.50
Isolate68	Fleet	68	3	15	3.67
Isolate68	CI11458	68	1	15	2.00
Isolate68	Algerian	68	2	15	2.67
Isolate68	Buloke	68	13	15	1.50
Isolate68	Corvette	68	9	15	4.00
Isolate68	Gilbert	68	12	15	4.50

!st leaf has fungal growth

Isolate68	Kombar	68	10	15	3.00	too young
Isolate68	Skiff	68	11	15	2.50	
Isolate68	Vlamingh	68	15	15	1.50	
Isolate68	Harbin	68	7	15	1.50	
Isolate68	Maritime	68	6	15	4.50	
Isolate68	Beecher	68	4	15	3.00	
Isolate68	Prior	68	8	15	3.00	1st leaf, 6
Isolate68	Rojo	68	14	15	2.00	
Isolate68	Yerong	68	5	15	2.00	
Isolate09	Rojo	9	14	16	4.00	
Isolate09	Kombar	9	10	16	7.50	
Isolate09	Beecher	9	4	16	8.00	Fungal growth
Isolate09	Skiff	9	11	16	3.50	
Isolate09	Vlamingh	9	15	16	2.50	
Isolate09	Harbin	9	7	16	3.50	
Isolate09	Prior	9	8	16	10.00	
Isolate09	Maritime	9	6	16	8.50	
Isolate09	Gilbert	9	12	16	9.00	
Isolate09	Yerong	9	5	16	7.33	
Isolate09	Fleet	9	3	16	9.00	
Isolate09	Corvette	9	9	16	8.00	
Isolate09	Buloke	9	13	16	3.00	
Isolate09	Algerian	9	2	16	6.50	
Isolate09	CI11458	9	1	16	5.00	
Isolate16	CI11458	16	1	17	2.50	
Isolate16	Vlamingh	16	15	17	2.50	
Isolate16	Prior	16	8	17	9.00	Fungal growth
Isolate16	Rojo	16	14	17	2.50	

Isolate16	Gilbert	16	12	17	6.00		Isolate45	CI11458	45	1	1	2.00
Isolate16	Fleet	16	3	17	7.00		Isolate45	Kombar	45	10	1	4.33
Isolate16	Harbin	16	7	17	3.50		Isolate45	Skiff	45	11	1	2.00
Isolate16	Skiff	16	11	17	5.00		Isolate45	Yerong	45	5	1	8.00
Isolate16	Yerong	16	5	17	7.67	Fungal growth	Isolate45	Algerian	45	2	1	2.00
Isolate16	Corvette	16	9	17	7.50		Isolate45	Gilbert	45	12	1	6.00
Isolate16	Algerian	16	2	17	5.50		Isolate45	Fleet	45	3	1	2.00
Isolate16	Beecher	16	4	17	8.00	Fungal growth	Isolate45	Prior	45	8	1	6.00
Isolate16	Kombar	16	10	17	3.50		Isolate45	Vlamingh	45	15	1	2.00
Isolate16	Maritime	16	6	17	7.67		Isolate45	Buloke	45	13	1	2.50
Isolate16	Buloke	16	13	17	2.50		Isolate45	Corvette	45	9	1	2.00
Isolate05	Maritime	5	6	18	3.00	too young and dry	Isolate45	Rojo	45	14	1	2.00
Isolate05	Harbin	5	7	18	2.67		Isolate45	Harbin	45	7	1	2.00
Isolate05	Rojo	5	14	18	3.00		NB29	Algerian	88	2	2	3.67
Isolate05	Vlamingh	5	15	18	1.50		NB29	Kombar	88	10	2	#DIV/0!
Isolate05	Corvette	5	9	18	5.33		NB29	Skiff	88	11	2	4.00
Isolate05	Algerian	5	2	18	4.00		NB29	Gilbert	88	12	2	4.33
Isolate05	Fleet	5	3	18	3.50		NB29	Prior	88	8	2	4.67
Isolate05	Buloke	5	13	18	3.00		NB29	Harbin	88	7	2	3.33
Isolate05	Prior	5	8	18	6.67	Clear reticulation	NB29	Maritime	88	6	2	9.00
Isolate05	CI11458	5	1	18	2.00		NB29	Vlamingh	88	15	2	3.00
Isolate05	Skiff	5	11	18	2.50		NB29	Yerong	88	5	2	7.00
Isolate05	Kombar	5	10	18	5.50		NB29	Rojo	88	14	2	2.50
Isolate05	Beecher	5	4	18	3.00		NB29	Fleet	88	3	2	5.00
Isolate05	Gilbert	5	12	18	4.00		NB29	Corvette	88	9	2	3.00
Isolate05	Yerong	5	5	18	7.00		NB29	Buloke	88	13	2	2.00
Isolate45	Beecher	45	4	1	4.00		NB29	Beecher	88	4	2	5.00
Isolate45	Maritime	45	6	1	5.50		NB29	CI11458	88	1	2	2.00

Plants didn't grow

NB85	Harbin	89	7	3	6.00
NB85	Beecher	89	4	3	3.50
NB85	CI11458	89	1	3	4.00
NB85	Vlamingh	89	15	3	3.00
NB85	Fleet	89	3	3	6.00
NB85	Skiff	89	11	3	7.33
NB85	Yerong	89	5	3	8.33
NB85	Gilbert	89	12	3	7.67
NB85	Buloke	89	13	3	6.33
NB85	Rojo	89	14	3	3.50
NB85	Maritime	89	6	3	#DIV/0!
NB85	Prior	89	8	3	10.00
NB85	Algerian	89	2	3	7.00
NB85	Corvette	89	9	3	9.33
NB85	Kombar	89	10	3	4.00
Isolate34	Gilbert	34	12	4	4.67
Isolate34	Yerong	34	5	4	5.67
Isolate34	Vlamingh	34	15	4	2.50
Isolate34	Harbin	34	7	4	7.00
Isolate34	Prior	34	8	4	6.33
Isolate34	Rojo	34	14	4	2.33
Isolate34	CI11458	34	1	4	3.50
Isolate34	Corvette	34	9	4	8.00
Isolate34	Algerian	34	2	4	5.00
Isolate34	Buloke	34	13	4	3.00
Isolate34	Beecher	34	4	4	7.00
Isolate34	Kombar	34	10	4	7.00
Isolate34	Skiff	34	11	4	3.33

Plants didn't grow

Isolate34	Fleet	34	3	4	7.33
Isolate34	Maritime	34	6	4	6.33
Isolate14	Vlamingh	14	15	5	3.00
Isolate14	Rojo	14	14	5	2.50
Isolate14	Algerian	14	2	5	4.00
Isolate14	Maritime	14	6	5	9.00
Isolate14	Prior	14	8	5	8.00
Isolate14	Harbin	14	7	5	2.00
Isolate14	Fleet	14	3	5	9.00
Isolate14	Skiff	14	11	5	3.00
Isolate14	Beecher	14	4	5	6.00
Isolate14	Kombar	14	10	5	5.00
Isolate14	Corvette	14	9	5	10.00
Isolate14	Buloke	14	13	5	2.50
Isolate14	CI11458	14	1	5	3.50
Isolate14	Yerong	14	5	5	5.00
Isolate14	Gilbert	14	12	5	5.50
Isolate36	Corvette	36	9	6	3.00
Isolate36	Skiff	36	11	6	2.50
Isolate36	Gilbert	36	12	6	3.00
Isolate36	Yerong	36	5	6	5.00
Isolate36	Kombar	36	10	6	4.33
Isolate36	Fleet	36	3	6	2.67
Isolate36	Algerian	36	2	6	3.67
Isolate36	Rojo	36	14	6	2.50
Isolate36	CI11458	36	1	6	2.50
Isolate36	Beecher	36	4	6	3.50
Isolate36	Vlamingh	36	15	6	4.00

Isolate36	Prior	36	8	6	4.50
Isolate36	Maritime	36	6	6	6.00
Isolate36	Harbin	36	7	6	3.00
Isolate36	Buloke	36	13	6	3.00
Isolate11	Kombar	11	10	7	4.67
Isolate11	Vlamingh	11	15	7	3.67
Isolate11	Gilbert	11	12	7	3.50
Isolate11	Skiff	11	11	7	3.00
Isolate11	Algerian	11	2	7	2.50
Isolate11	Prior	11	8	7	5.00
Isolate11	Harbin	11	7	7	2.50
Isolate11	Yerong	11	5	7	2.50
Isolate11	Corvette	11	9	7	6.00
Isolate11	Buloke	11	13	7	2.00
Isolate11	Maritime	11	6	7	2.00
Isolate11	Rojo	11	14	7	2.00
Isolate11	Beecher	11	4	7	2.00
Isolate11	CI11458	11	1	7	2.00
Isolate11	Fleet	11	3	7	2.00
Isolate08	Rojo	8	14	8	2.00
Isolate08	Yerong	8	5	8	7.00
Isolate08	Beecher	8	4	8	6.00
Isolate08	Vlamingh	8	15	8	2.00
Isolate08	CI11458	8	1	8	8.33
Isolate08	Harbin	8	7	8	7.00
Isolate08	Gilbert	8	12	8	2.00
Isolate08	Buloke	8	13	8	2.00
Isolate08	Kombar	8	10	8	7.00

Isolate08	Prior	8	8	8	6.50
Isolate08	Fleet	8	3	8	3.00
Isolate08	Algerian	8	2	8	3.50
Isolate08	Skiff	8	11	8	3.00
Isolate08	Maritime	8	6	8	6.00
Isolate08	Corvette	8	9	8	3.50
Isolate47	Gilbert	47	12	9	2.50
Isolate47	Harbin	47	7	9	2.50
Isolate47	Fleet	47	3	9	3.00
Isolate47	Corvette	47	9	9	5.67
Isolate47	Buloke	47	13	9	6.50
Isolate47	Vlamingh	47	15	9	3.00
Isolate47	Skiff	47	11	9	2.50
Isolate47	CI11458	47	1	9	4.00
Isolate47	Rojo	47	14	9	1.00
Isolate47	Beecher	47	4	9	2.00
Isolate47	Algerian	47	2	9	2.00
Isolate47	Yerong	47	5	9	#DIV/0!
Isolate47	Prior	47	8	9	#DIV/0!
Isolate47	Kombar	47	10	9	#DIV/0!
Isolate47	Maritime	47	6	9	#DIV/0!
Isolate68	Buloke	68	13	10	2.00
Isolate68	Corvette	68	9	10	2.00
Isolate68	Vlamingh	68	15	10	2.00
Isolate68	Beecher	68	4	10	2.00
Isolate68	Rojo	68	14	10	2.00
Isolate68	CI11458	68	1	10	2.00
Isolate68	Kombar	68	10	10	2.00

Plants didn't grow
Plants didn't grow
Plants didn't grow
Plants didn't grow

Isolate68	Harbin	68	7	10	2.00
Isolate68	Prior	68	8	10	2.00
Isolate68	Maritime	68	6	10	2.00
Isolate68	Gilbert	68	12	10	2.00
Isolate68	Skiff	68	11	10	2.00
Isolate68	Fleet	68	3	10	2.00
Isolate68	Algerian	68	2	10	2.00
Isolate68	Yerong	68	5	10	2.00
Isolate22	Prior	22	8	11	9.00
Isolate22	Corvette	22	9	11	6.00
Isolate22	Fleet	22	3	11	3.00
Isolate22	Algerian	22	2	11	3.67
Isolate22	Maritime	22	6	11	5.00
Isolate22	Gilbert	22	12	11	4.00
Isolate22	Rojo	22	14	11	2.00
Isolate22	Kombar	22	10	11	4.00
Isolate22	Beecher	22	4	11	3.00
Isolate22	Yerong	22	5	11	6.00
Isolate22	Skiff	22	11	11	4.00
Isolate22	Harbin	22	7	11	2.50
Isolate22	Buloke	22	13	11	2.50
Isolate22	CI11458	22	1	11	3.00
Isolate22	Vlamingh	22	15	11	3.00
Isolate13	Skiff	13	11	12	2.00
Isolate13	Algerian	13	2	12	2.00
Isolate13	Buloke	13	13	12	3.00
Isolate13	Yerong	13	5	12	2.00
Isolate13	CI11458	13	1	12	2.00

Isolate13	Kombar	13	10	12	5.00
Isolate13	Harbin	13	7	12	5.00
Isolate13	Maritime	13	6	12	7.33
Isolate13	Prior	13	8	12	2.00
Isolate13	Vlamingh	13	15	12	2.00
Isolate13	Corvette	13	9	12	2.00
Isolate13	Fleet	13	3	12	2.00
Isolate13	Beecher	13	4	12	3.00
Isolate13	Gilbert	13	12	12	2.00
Isolate13	Rojo	13	14	12	2.00
Isolate40	Maritime	40	6	13	2.00
Isolate40	Fleet	40	3	13	3.00
Isolate40	CI11458	40	1	13	3.00
Isolate40	Beecher	40	4	13	3.00
Isolate40	Rojo	40	14	13	3.00
Isolate40	Gilbert	40	12	13	3.00
Isolate40	Yerong	40	5	13	3.00
Isolate40	Buloke	40	13	13	3.00
Isolate40	Prior	40	8	13	3.00
Isolate40	Skiff	40	11	13	3.00
Isolate40	Harbin	40	7	13	3.00
Isolate40	Algerian	40	2	13	3.00
Isolate40	Vlamingh	40	15	13	3.00
Isolate40	Kombar	40	10	13	3.00
Isolate40	Corvette	40	9	13	3.00
Isolate64	Prior	64	8	14	2.00
Isolate64	Yerong	64	5	14	2.50
Isolate64	Beecher	64	4	14	3.00

Isolate64	Vlamingh	64	15	14	2.00
Isolate64	Corvette	64	9	14	2.00
Isolate64	Buloke	64	13	14	2.00
Isolate64	Kombar	64	10	14	2.00
Isolate64	Harbin	64	7	14	2.00
Isolate64	Maritime	64	6	14	4.00
Isolate64	CI11458	64	1	14	2.00
Isolate64	Rojo	64	14	14	2.00
Isolate64	Gilbert	64	12	14	2.00
Isolate64	Algerian	64	2	14	2.00
Isolate64	Fleet	64	3	14	2.00
Isolate64	Skiff	64	11	14	2.00
Isolate04	CI11458	4	1	15	3.00
Isolate04	Prior	4	8	15	2.00
Isolate04	Corvette	4	9	15	2.00
Isolate04	Fleet	4	3	15	2.00
Isolate04	Buloke	4	13	15	3.00
Isolate04	Rojo	4	14	15	3.00
Isolate04	Vlamingh	4	15	15	3.00
Isolate04	Algerian	4	2	15	3.00
Isolate04	Maritime	4	6	15	3.00
Isolate04	Harbin	4	7	15	3.00
Isolate04	Beecher	4	4	15	3.00
Isolate04	Skiff	4	11	15	3.00
Isolate04	Gilbert	4	12	15	3.00
Isolate04	Yerong	4	5	15	3.00
Isolate04	Kombar	4	10	15	3.00
Isolate62	Fleet	62	3	16	5.33
Isolate62	Buloke	62	13	16	5.33
Isolate62	Algerian	62	2	16	5.67
Isolate62	Corvette	62	9	16	7.67
Isolate62	Maritime	62	6	16	6.67
Isolate62	Beecher	62	4	16	7.00
Isolate62	CI11458	62	1	16	3.00
Isolate62	Skiff	62	11	16	3.33
Isolate62	Prior	62	8	16	7.67
Isolate62	Kombar	62	10	16	5.67
Isolate62	Yerong	62	5	16	6.67
Isolate62	Gilbert	62	12	16	4.67
Isolate62	Harbin	62	7	16	3.33
Isolate62	Rojo	62	14	16	3.67
Isolate62	Vlamingh	62	15	16	2.00
Isolate30	Beecher	30	4	17	3.50
Isolate30	Maritime	30	6	17	7.00
Isolate30	CI11458	30	1	17	2.50
Isolate30	Kombar	30	10	17	5.50
Isolate30	Skiff	30	11	17	4.67
Isolate30	Yerong	30	5	17	5.33
Isolate30	Algerian	30	2	17	6.00
Isolate30	Gilbert	30	12	17	4.00
Isolate30	Fleet	30	3	17	3.00
Isolate30	Prior	30	8	17	8.00
Isolate30	Vlamingh	30	15	17	2.50
Isolate30	Buloke	30	13	17	3.00
Isolate30	Corvette	30	9	17	5.00
Isolate30	Rojo	30	14	17	3.00

Isolate30	Harbin	30	7	17	3.00

Isolate	Genotype	Iso	var	serial	Day 14	Comments
NB85	CI11458	89	1	1	1.50	
NB85	Fleet	89	3	1	1.50	
NB85	Corvette	89	9	1	3.67	
NB85	Harbin	89	7	1	1.00	
NB85	Vlamingh	89	15	1	1.00	
NB85	Algerian	89	2	1	1.00	
NB85	Kombar	89	10	1	1.67	
NB85	Buloke	89	13	1	1.50	
NB85	Yerong	89	5	1	1.00	
NB85	Gilbert	89	12	1	1.00	
NB85	Beecher	89	4	1	1.00	
NB85	Rojo	89	14	1	1.00	
NB85	Maritime	89	6	1	3.33	
NB85	Prior	89	8	1	1.00	
NB85	Skiff	89	11	1	1.00	
Isolate04	Buloke	4	13	2	1.00	
Isolate04	Harbin	4	7	2	1.00	
Isolate04	Rojo	4	14	2	1.50	
Isolate04	Yerong	4	5	2	3.00	
Isolate04	Gilbert	4	12	2	6.00	
Isolate04	Skiff	4	11	2	1.00	
Isolate04	Corvette	4	9	2	5.67	
Isolate04	Fleet	4	3	2	3.33	
Isolate04	CI11458	4	1	2	1.67	
Isolate04	Prior	4	8	2	1.00	
Isolate04	Kombar	4	10	2	4.33	
Isolate04	Maritime	4	6	2	3.00	
Isolate04	Vlamingh	4	15	2	1.00	
Isolate04	Beecher	4	4	2	6.00	
Isolate04	Algerian	4	2	2	2.00	
Isolate22	CI11458	22	1	4	3.50	
Isolate22	Algerian	22	2	4	4.50	
Isolate22	Maritime	22	6	4	2.50	
Isolate22	Gilbert	22	12	4	4.00	
Isolate22	Vlamingh	22	15	4	1.00	
Isolate22	Prior	22	8	4	2.00	
Isolate22	Yerong	22	5	4	2.00	
Isolate22	Beecher	22	4	4	2.00	
Isolate22	Fleet	22	3	4	3.50	
Isolate22	Rojo	22	14	4	1.00	
Isolate22	Corvette	22	9	4	4.00	
Isolate22	Kombar	22	10	4	1.50	
Isolate22	Harbin	22	7	4	1.00	
Isolate22	Skiff	22	11	4	2.00	
Isolate22	Buloke	22	13	4	3.33	
Isolate54	Skiff	54	11	6	1.00	
Isolate54	Prior	54	8	6	5.00	
Isolate54	Corvette	54	9	6	7.00	
Isolate54	Rojo	54	14	6	1.00	
Isolate54	Vlamingh	54	15	6	1.00	
Isolate54	Gilbert	54	12	6	5.00	

Isolate54	Fleet	54	3	6	5.50
Isolate54	CI11458	54	1	6	6.00
Isolate54	Harbin	54	7	6	4.00
Isolate54	Algerian	54	2	6	6.00
Isolate54	Maritime	54	6	6	5.00
Isolate54	Yerong	54	5	6	1.00
Isolate54	Beecher	54	4	6	5.00
Isolate54	Buloke	54	13	6	2.00
Isolate54	Kombar	54	10	6	3.00
NB29	Gilbert	88	12	7	3.00
NB29	Buloke	88	13	7	4.00
NB29	Algerian	88	2	7	1.00
NB29	Skiff	88	11	7	1.00
NB29	Harbin	88	7	7	1.00
NB29	Maritime	88	6	7	3.00
NB29	Corvette	88	9	7	3.00
NB29	Rojo	88	14	7	1.00
NB29	Kombar	88	10	7	1.00
NB29	Beecher	88	4	7	5.50
NB29	Yerong	88	5	7	1.50
NB29	Prior	88	8	7	2.00
NB29	Vlamingh	88	15	7	3.00
NB29	CI11458	88	1	7	2.00
NB29	Fleet	88	3	7	1.00
Isolate68	Kombar	68	10	8	1.00
Isolate68	Rojo	68	14	8	1.00
Isolate68	Fleet	68	3	8	4.50
Isolate68	Algerian	68	2	8	1.00
Isolate68	Prior	68	8	8	3.00
Isolate68	Beecher	68	4	8	4.00
Isolate68	Maritime	68	6	8	6.67
Isolate68	Yerong	68	5	8	2.00
Isolate68	Vlamingh	68	15	8	1.00
Isolate68	CI11458	68	1	8	3.00
Isolate68	Buloke	68	13	8	4.00
Isolate68	Harbin	68	7	8	1.00
Isolate68	Skiff	68	11	8	1.50
Isolate68	Corvette	68	9	8	6.33
Isolate68	Gilbert	68	12	8	3.00
Isolate28	Maritime	28	6	9	3.50
Isolate28	Kombar	28	10	9	3.50
Isolate28	Gilbert	28	12	9	4.00
Isolate28	Buloke	28	13	9	5.00
Isolate28	Beecher	28	4	9	3.00
Isolate28	CI11458	28	1	9	3.50
Isolate28	Rojo	28	14	9	1.50
Isolate28	Prior	28	8	9	5.00
Isolate28	Vlamingh	28	15	9	1.00
Isolate28	Harbin	28	7	9	3.00
Isolate28	Algerian	28	2	9	3.00
Isolate28	Skiff	28	11	9	3.00
Isolate28	Fleet	28	3	9	5.00
Isolate28	Corvette	28	9	9	7.00
Isolate28	Yerong	28	5	9	1.00
Isolate11	Prior	11	8	10	3.00
Isolate11	Algerian	11	2	10	2.00

Isolate11	Skiff	11	11	10	1.00
Isolate11	Maritime	11	6	10	2.00
Isolate11	Corvette	11	9	10	6.33
Isolate11	Fleet	11	3	10	1.50
Isolate11	Gilbert	11	12	10	2.00
Isolate11	Harbin	11	7	10	2.33
Isolate11	Beecher	11	4	10	1.50
Isolate11	Vlamingh	11	15	10	1.00
Isolate11	Yerong	11	5	10	2.00
Isolate11	Buloke	11	13	10	1.00
Isolate11	CI11458	11	1	10	5.00
Isolate11	Kombar	11	10	10	1.50
Isolate11	Rojo	11	14	10	1.00
Isolate36	Prior	36	8	11	3.50
Isolate36	Rojo	36	14	11	1.00
Isolate36	Harbin	36	7	11	1.00
Isolate36	Yerong	36	5	11	2.00
Isolate36	Skiff	36	11	11	1.00
Isolate36	Kombar	36	10	11	2.00
Isolate36	Vlamingh	36	15	11	2.00
Isolate36	Corvette	36	9	11	6.00
Isolate36	CI11458	36	1	11	4.00
Isolate36	Gilbert	36	12	11	5.67
Isolate36	Buloke	36	13	11	4.67
Isolate36	Algerian	36	2	11	3.00
Isolate36	Fleet	36	3	11	5.00
Isolate36	Maritime	36	6	11	5.33
Isolate36	Beecher	36	4	11	3.33

Isolate55	Kombar	55	10	12	2.00	
Isolate55	Skiff	55	11	12	1.00	
Isolate55	Vlamingh	55	15	12	2.50	
Isolate55	Harbin	55	7	12	2.67	
Isolate55	Beecher	55	4	12	5.50	
Isolate55	Corvette	55	9	12	5.00	
Isolate55	Buloke	55	13	12	1.50	
Isolate55	Gilbert	55	12	12	3.00	
Isolate55	Maritime	55	6	12	2.67	
Isolate55	Fleet	55	3	12	4.67	
Isolate55	CI11458	55	1	12	1.50	
Isolate55	Prior	55	8	12	5.50	very consistent
Isolate55	Yerong	55	5	12	2.00	
Isolate55	Rojo	55	14	12	2.50	
Isolate55	Algerian	55	2	12	1.00	
Isolate35	Harbin	35	7	13	2.00	
Isolate35	Kombar	35	10	13	4.67	
Isolate35	Vlamingh	35	15	13	2.50	
Isolate35	Corvette	35	9	13	5.50	
Isolate35	Prior	35	8	13	4.00	
Isolate35	Buloke	35	13	13	1.50	
Isolate35	Beecher	35	4	13	4.00	
Isolate35	Yerong	35	5	13	2.00	
Isolate35	Maritime	35	6	13	1.50	
Isolate35	Rojo	35	14	13	1.50	
Isolate35	CI11458	35	1	13	2.33	
Isolate35	Skiff	35	11	13	1.00	
Isolate35	Algerian	35	2	13	1.00	

Isolate35	Gilbert	35	12	13	3.50
Isolate35	Fleet	35	3	13	3.67
Isolate30	Buloke	30	13	14	4.50
Isolate30	Fleet	30	3	14	1.00
Isolate30	Algerian	30	2	14	1.00
Isolate30	Beecher	30	4	14	1.00
Isolate30	Maritime	30	6	14	2.50
Isolate30	Yerong	30	5	14	1.00
Isolate30	Kombar	30	10	14	1.00
Isolate30	Rojo	30	14	14	1.00
Isolate30	Vlamingh	30	15	14	1.00
Isolate30	Corvette	30	9	14	3.00
Isolate30	Gilbert	30	12	14	3.50
Isolate30	Harbin	30	7	14	1.00
Isolate30	Skiff	30	11	14	2.00
Isolate30	Prior	30	8	14	2.50
Isolate30	CI11458	30	1	14	2.00
Isolate13	CI11458	13	1	15	2.00
Isolate13	Maritime	13	6	15	3.00
Isolate13	Corvette	13	9	15	3.00
Isolate13	Buloke	13	13	15	2.00
Isolate13	Vlamingh	13	15	15	1.00
Isolate13	Rojo	13	14	15	1.50
Isolate13	Skiff	13	11	15	1.00
Isolate13	Fleet	13	3	15	2.00
Isolate13	Beecher	13	4	15	2.00
Isolate13	Kombar	13	10	15	1.00
Isolate13	Algerian	13	2	15	2.00
Isolate13	Gilbert	13	12	15	1.50
Isolate13	Yerong	13	5	15	1.00
Isolate13	Harbin	13	7	15	1.00
Isolate13	Prior	13	8	15	1.00
Isolate41	Beecher	41	4	16	7.00
Isolate41	Vlamingh	41	15	16	1.00
Isolate41	Skiff	41	11	16	2.00
Isolate41	CI11458	41	1	16	3.00
Isolate41	Corvette	41	9	16	6.00
Isolate41	Yerong	41	5	16	2.00
Isolate41	Prior	41	8	16	3.50
Isolate41	Harbin	41	7	16	1.67
Isolate41	Gilbert	41	12	16	3.67
Isolate41	Rojo	41	14	16	2.00
Isolate41	Maritime	41	6	16	6.33
Isolate41	Fleet	41	3	16	4.00
Isolate41	Algerian	41	2	16	2.50
Isolate41	Buloke	41	13	16	3.00
Isolate41	Kombar	41	10	16	3.67
Isolate07	CI11458	7	1	17	4.50
Isolate07	Fleet	7	3	17	2.67
Isolate07	Corvette	7	9	17	4.00
Isolate07	Harbin	7	7	17	2.50
Isolate07	Vlamingh	7	15	17	2.00
Isolate07	Algerian	7	2	17	2.50
Isolate07	Kombar	7	10	17	3.00
Isolate07	Buloke	7	13	17	2.50
Isolate07	Yerong	7	5	17	2.50

Isolate07	Gilbert	7	12	17	3.33	
Isolate07	Beecher	7	4	17	4.00	
Isolate07	Rojo	7	14	17	2.33	
Isolate07	Maritime	7	6	17	3.33	
Isolate07	Prior	7	8	17	3.33	
Isolate07	Skiff	7	11	17	3.00	
Isolate09	Buloke	9	13	18	4.67	
Isolate09	Harbin	9	7	18	1.67	
Isolate09	Rojo	9	14	18	3.50	
Isolate09	Yerong	9	5	18	1.00	1st leaf, 6
Isolate09	Gilbert	9	12	18	4.00	
Isolate09	Skiff	9	11	18	1.00	
Isolate09	Corvette	9	9	18	6.33	
Isolate09	Fleet	9	3	18	5.00	
Isolate09	CI11458	9	1	18	2.00	1st leaf, 6
Isolate09	Prior	9	8	18	4.67	
Isolate09	Kombar	9	10	18	1.00	1st leaf, 4
Isolate09	Maritime	9	6	18	3.00	
Isolate09	Vlamingh	9	15	18	1.00	
Isolate09	Beecher	9	4	18	4.33	
Isolate09	Algerian	9	2	18	2.50	
Isolate21	Kombar	21	10	1	6.00	
Isolate21	Harbin	21	7	1	5.00	
Isolate21	CI11458	21	1	1	2.00	
Isolate21	Corvette	21	9	1	7.50	
Isolate21	Buloke	21	13	1	3.50	
Isolate21	Fleet	21	3	1	5.50	
Isolate21	Yerong	21	5	1	6.00	welting

Isolate21	Gilbert	21	12	1	4.00	
Isolate21	Algerian	21	2	1	5.00	
Isolate21	Beecher	21	4	1	8.00	
Isolate21	Skiff	21	11	1	4.50	
Isolate21	Prior	21	8	1	7.50	
Isolate21	Rojo	21	14	1	4.00	
Isolate21	Maritime	21	6	1	7.00	
Isolate21	Vlamingh	21	15	1	3.00	
Isolate34	Skiff	34	11	2	2.50	
Isolate34	Corvette	34	9	2	4.50	
Isolate34	Buloke	34	13	2	2.00	
Isolate34	Algerian	34	2	2	5.00	
Isolate34	Yerong	34	5	2	7.00	
Isolate34	Rojo	34	14	2	2.00	
Isolate34	Beecher	34	4	2	6.00	
Isolate34	Maritime	34	6	2	7.00	
Isolate34	Fleet	34	3	2	4.50	
Isolate34	CI11458	34	1	2	2.00	
Isolate34	Vlamingh	34	15	2	2.00	
Isolate34	Harbin	34	7	2	2.00	1st leaf, 6
Isolate34	Gilbert	34	12	2	3.00	welting
Isolate34	Prior	34	8	2	3.00	
Isolate34	Kombar	34	10	2	3.50	1st leaf, 7
NB85	Gilbert	89	12	3	7.50	
NB85	Rojo	89	14	3	2.00	
NB85	Skiff	89	11	3	4.00	
NB85	Algerian	89	2	3	7.00	
NB85	Prior	89	8	3	7.00	

NB85	Beecher	89	4	3	4.00	
NB85	Buloke	89	13	3	5.50	1st leaf, 7
NB85	Yerong	89	5	3	2.00	
NB85	Kombar	89	10	3	2.00	
NB85	Maritime	89	6	3	4.50	
NB85	Fleet	89	3	3	5.50	
NB85	Harbin	89	7	3	5.50	
NB85	Vlamingh	89	15	3	2.00	
NB85	CI11458	89	1	3	2.00	
NB85	Corvette	89	9	3	8.00	
Isolate62	Yerong	62	5	5	2.00	
Isolate62	Harbin	62	7	5	6.00	
Isolate62	Rojo	62	14	5	2.00	
Isolate62	Prior	62	8	5	2.00	
Isolate62	Kombar	62	10	5	5.50	
Isolate62	Gilbert	62	12	5	2.00	
Isolate62	Skiff	62	11	5	2.50	
Isolate62	Beecher	62	4	5	2.00	
Isolate62	Algerian	62	2	5	2.00	
Isolate62	Fleet	62	3	5	2.00	
Isolate62	Vlamingh	62	15	5	3.00	
Isolate62	Maritime	62	6	5	4.50	
Isolate62	Corvette	62	9	5	4.00	
Isolate62	Buloke	62	13	5	2.00	
Isolate62	CI11458	62	1	5	2.00	
Isolate44	Vlamingh	44	15	6	4.00	
Isolate44	Kombar	44	10	6	2.00	1st leaf, 5
Isolate44	Algerian	44	2	6	2.00	
Isolate44	Beecher	44	4	6	2.00	
Isolate44	Rojo	44	14	6	2.00	welting
Isolate44	Fleet	44	3	6	2.00	
Isolate44	Prior	44	8	6	8.00	
Isolate44	Maritime	44	6	6	4.00	
Isolate44	Corvette	44	9	6	2.00	
Isolate44	Buloke	44	13	6	3.50	
Isolate44	CI11458	44	1	6	3.50	
Isolate44	Gilbert	44	12	6	2.00	
Isolate44	Yerong	44	5	6	3.50	
Isolate44	Harbin	44	7	6	3.50	
Isolate44	Skiff	44	11	6	3.50	
Isolate40	Skiff	40	11	7	5.00	
Isolate40	Vlamingh	40	15	7	2.00	
Isolate40	Gilbert	40	12	7	6.50	
Isolate40	CI11458	40	1	7	5.50	
Isolate40	Yerong	40	5	7	4.50	
Isolate40	Buloke	40	13	7	4.00	
Isolate40	Algerian	40	2	7	4.00	
Isolate40	Corvette	40	9	7	7.00	
Isolate40	Harbin	40	7	7	6.50	
Isolate40	Prior	40	8	7	4.50	
Isolate40	Fleet	40	3	7	4.50	
Isolate40	Rojo	40	14	7	5.00	
Isolate40	Maritime	40	6	7	4.00	
Isolate40	Kombar	40	10	7	3.00	
Isolate40	Beecher	40	4	7	3.50	
Isolate16	CI11458	16	1	8	7.00	

Isolate16	Rojo	16	14	8	4.00	
Isolate16	Corvette	16	9	8	7.00	
Isolate16	Maritime	16	6	8	8.00	
Isolate16	Prior	16	8	8	7.50	
Isolate16	Harbin	16	7	8	4.00	
Isolate16	Yerong	16	5	8	8.00	
Isolate16	Beecher	16	4	8	7.50	
Isolate16	Vlamingh	16	15	8	3.00	
Isolate16	Kombar	16	10	8	2.50	
Isolate16	Buloke	16	13	8	4.50	
Isolate16	Gilbert	16	12	8	2.50	
Isolate16	Skiff	16	11	8	2.00	
Isolate16	Algerian	16	2	8	#DIV/0!	no growth
Isolate16	Fleet	16	3	8	2.00	
NB29	Prior	88	8	9	6.50	
NB29	Rojo	88	14	9	2.00	
NB29	Gilbert	88	12	9	4.50	
NB29	Beecher	88	4	9	8.00	
NB29	Maritime	88	6	9	7.00	
NB29	CI11458	88	1	9	4.50	
NB29	Vlamingh	88	15	9	4.00	
NB29	Buloke	88	13	9	3.00	
NB29	Skiff	88	11	9	4.00	
NB29	Corvette	88	9	9	6.50	
NB29	Kombar	88	10	9	8.50	
NB29	Fleet	88	3	9	4.00	
NB29	Harbin	88	7	9	3.50	
NB29	Algerian	88	2	9	6.00	
NB29	Yerong	88	5	9	6.00	
Isolate30	Fleet	30	3	10	3.50	
Isolate30	Yerong	30	5	10	5.50	
Isolate30	Beecher	30	4	10	4.00	
Isolate30	Harbin	30	7	10	3.00	
Isolate30	Gilbert	30	12	10	6.00	
Isolate30	Vlamingh	30	15	10	3.00	
Isolate30	Prior	30	8	10	10.00	totally dry
Isolate30	Rojo	30	14	10	5.00	
Isolate30	Kombar	30	10	10	8.00	
Isolate30	Buloke	30	13	10	9.00	
Isolate30	Maritime	30	6	10	2.50	
Isolate30	Algerian	30	2	10	8.00	
Isolate30	Skiff	30	11	10	5.00	
Isolate30	Corvette	30	9	10	6.50	
Isolate30	CI11458	30	1	10	4.00	
Isolate14	Vlamingh	14	15	11	3.50	
Isolate14	Yerong	14	5	11	4.00	
Isolate14	Prior	14	8	11	6.00	
Isolate14	Buloke	14	13	11	3.00	
Isolate14	CI11458	14	1	11	3.00	
Isolate14	Maritime	14	6	11	4.50	
Isolate14	Fleet	14	3	11	4.50	
Isolate14	Beecher	14	4	11	6.50	
Isolate14	Harbin	14	7	11	3.00	
Isolate14	Algerian	14	2	11	2.00	
Isolate14	Gilbert	14	12	11	3.50	
Isolate14	Corvette	14	9	11	4.00	

Isolate14	Kombar	14	10	11	3.00
Isolate14	Rojo	14	14	11	2.00
Isolate14	Skiff	14	11	11	3.00
Isolate64	Algerian	64	2	12	7.00
Isolate64	Fleet	64	3	12	6.00
Isolate64	Kombar	64	10	12	6.00
Isolate64	Rojo	64	14	12	4.00
Isolate64	Vlamingh	64	15	12	3.00
Isolate64	Skiff	64	11	12	6.00
Isolate64	Corvette	64	9	12	8.00
Isolate64	Gilbert	64	12	12	5.50
Isolate64	Maritime	64	6	12	7.00
Isolate64	Beecher	64	4	12	6.00
Isolate64	CI11458	64	1	12	4.50
Isolate64	Yerong	64	5	12	2.00
Isolate64	Prior	64	8	12	5.00
Isolate64	Harbin	64	7	12	2.50
Isolate64	Buloke	64	13	12	2.00
Isolate28	Buloke	28	13	13	3.50
Isolate28	Beecher	28	4	13	3.50
Isolate28	Corvette	28	9	13	6.50
Isolate28	Yerong	28	5	13	8.00
Isolate28	Skiff	28	11	13	5.50
Isolate28	Maritime	28	6	13	2.00
Isolate28	Fleet	28	3	13	6.00
Isolate28	CI11458	28	1	13	2.50
Isolate28	Vlamingh	28	15	13	2.00
Isolate28	Rojo	28	14	13	2.00

Isolate28	Harbin	28	7	13	4.50
Isolate28	Algerian	28	2	13	3.50
Isolate28	Gilbert	28	12	13	3.50
Isolate28	Prior	28	8	13	3.00
Isolate28	Kombar	28	10	13	2.00
Isolate08	Maritime	8	6	14	2.00
Isolate08	Skiff	8	11	14	2.00
Isolate08	Fleet	8	3	14	2.00
Isolate08	Buloke	8	13	14	2.00
Isolate08	Algerian	8	2	14	2.00
Isolate08	CI11458	8	1	14	2.00
Isolate08	Gilbert	8	12	14	2.00
Isolate08	Kombar	8	10	14	2.00
Isolate08	Harbin	8	7	14	2.00
Isolate08	Yerong	8	5	14	2.00
Isolate08	Corvette	8	9	14	2.00
Isolate08	Prior	8	8	14	2.00
Isolate08	Vlamingh	8	15	14	2.00
Isolate08	Beecher	8	4	14	2.00
Isolate08	Rojo	8	14	14	2.00
Isolate38	Prior	38	8	15	5.00
Isolate38	Fleet	38	3	15	5.50
Isolate38	Buloke	38	13	15	2.00
Isolate38	Vlamingh	38	15	15	3.50
Isolate38	Kombar	38	10	15	2.00
Isolate38	Skiff	38	11	15	2.00
Isolate38	CI11458	38	1	15	7.00
Isolate38	Rojo	38	14	15	3.00

1st leaf, 6-7

Isolate38	Gilbert	38	12	15	4.00
Isolate38	Maritime	38	6	15	8.00
Isolate38	Algerian	38	2	15	6.00
Isolate38	Beecher	38	4	15	5.50
Isolate38	Harbin	38	7	15	2.50
Isolate38	Corvette	38	9	15	2.50
Isolate38	Yerong	38	5	15	5.00
Isolate05	Harbin	5	7	16	3.00
Isolate05	Algerian	5	2	16	2.00
Isolate05	Yerong	5	5	16	2.00
Isolate05	Gilbert	5	12	16	4.00
Isolate05	Fleet	5	3	16	5.00
Isolate05	Beecher	5	4	16	3.00
Isolate05	Maritime	5	6	16	5.50
Isolate05	Prior	5	8	16	6.50
Isolate05	Kombar	5	10	16	3.00
Isolate05	Corvette	5	9	16	3.00
Isolate05	Skiff	5	11	16	3.00
Isolate05	CI11458	5	1	16	3.00
Isolate05	Rojo	5	14	16	2.50
Isolate05	Buloke	5	13	16	2.50
Isolate05	Vlamingh	5	15	16	2.50
Isolate23	Kombar	23	10	17	4.50
Isolate23	Harbin	23	7	17	2.00
Isolate23	CI11458	23	1	17	3.00
Isolate23	Corvette	23	9	17	6.00
Isolate23	Buloke	23	13	17	5.00
Isolate23	Fleet	23	3	17	2.00
Isolate23	Yerong	23	5	17	2.00
Isolate23	Gilbert	23	12	17	4.50
Isolate23	Algerian	23	2	17	4.00
Isolate23	Beecher	23	4	17	7.00
Isolate23	Skiff	23	11	17	5.50
Isolate23	Prior	23	8	17	4.50
Isolate23	Rojo	23	14	17	3.00
Isolate23	Maritime	23	6	17	5.00
Isolate23	Vlamingh	23	15	17	3.00
Isolate65	Skiff	65	11	18	2.00
Isolate65	Corvette	65	9	18	2.00
Isolate65	Buloke	65	13	18	2.00
Isolate65	Algerian	65	2	18	2.00
Isolate65	Yerong	65	5	18	2.00
Isolate65	Rojo	65	14	18	2.00
Isolate65	Beecher	65	4	18	2.00
Isolate65	Maritime	65	6	18	2.00
Isolate65	Fleet	65	3	18	3.00
Isolate65	CI11458	65	1	18	2.00
Isolate65	Vlamingh	65	15	18	2.00
Isolate65	Harbin	65	7	18	2.00
Isolate65	Gilbert	65	12	18	2.00
Isolate65	Prior	65	8	18	2.00
Isolate65	Kombar	65	10	18	2.00
NB29	Maritime	88	6	2	9.50
NB29	Harbin	88	7	2	4.50
NB29	Rojo	88	14	2	3.50
NB29	Vlamingh	88	15	2	2.50

NB29	Corvette	88	9	2	9.50	
NB29	Algerian	88	2	2	4.50	
NB29	Fleet	88	3	2	7.00	Fungal growth
NB29	Buloke	88	13	2	3.00	
NB29	Prior	88	8	2	5.50	
NB29	CI11458	88	1	2	4.50	
NB29	Skiff	88	11	2	4.00	
NB29	Kombar	88	10	2	0.00	, 2nd leaf barel germinated_1st leaf, 6-7
NB29	Beecher	88	4	2	7.50	
NB29	Gilbert	88	12	2	4.00	
NB29	Yerong	88	5	2	2.00	1st leaf, 4
Isolate70	Algerian	70	2	3	3.50	
Isolate70	Buloke	70	13	3	3.50	
Isolate70	Rojo	70	14	3	2.00	
Isolate70	Beecher	70	4	3	2.50	
Isolate70	Fleet	70	3	3	2.33	
Isolate70	Skiff	70	11	3	2.50	
Isolate70	Gilbert	70	12	3	3.00	
Isolate70	Kombar	70	10	3	3.00	
Isolate70	CI11458	70	1	3	2.50	
Isolate70	Prior	70	8	3	5.00	
Isolate70	Yerong	70	5	3	3.00	
Isolate70	Vlamingh	70	15	3	2.00	
Isolate70	Maritime	70	6	3	2.00	
Isolate70	Corvette	70	9	3	5.00	
Isolate70	Harbin	70	7	3	1.00	
control	Prior	54	8	4	0.00	
control	Vlamingh	54	15	4	0.00	
control	Corvette	54	9	4	0.00	
control	Harbin	54	7	4	0.00	
control	CI11458	54	1	4	0.00	
control	Buloke	54	13	4	0.00	
control	Algerian	54	2	4	0.00	
control	Yerong	54	5	4	0.00	
control	Maritime	54	6	4	0.00	
control	Gilbert	54	12	4	0.00	
control	Beecher	54	4	4	0.00	
control	Fleet	54	3	4	0.00	
control	Kombar	54	10	4	0.00	
control	Rojo	54	14	4	0.00	
control	Skiff	54	11	4	0.00	
NB85	Rojo	89	14	5	3.00	
NB85	Vlamingh	89	15	5	3.00	
NB85	Buloke	89	13	5	4.00	
NB85	Corvette	89	9	5	7.00	Fungal growth
NB85	Kombar	89	10	5	3.50	
NB85	Skiff	89	11	5	5.50	
NB85	Beecher	89	4	5	4.00	
NB85	Harbin	89	7	5	4.67	
NB85	Prior	89	8	5	7.00	Fungal growth
NB85	Yerong	89	5	5	6.00	
NB85	Algerian	89	2	5	6.50	
NB85	Maritime	89	6	5	4.50	
NB85	CI11458	89	1	5	3.00	
NB85	Gilbert	89	12	5	4.50	

NB85	Fleet	89	3	5	5.50
Isolate38	Harbin	38	7	6	3.50
Isolate38	Kombar	38	10	6	5.50
Isolate38	Yerong	38	5	6	4.00
Isolate38	Vlamingh	38	15	6	2.50
Isolate38	Maritime	38	6	6	6.00
Isolate38	Fleet	38	3	6	6.00
Isolate38	Algerian	38	2	6	6.00
Isolate38	Gilbert	38	12	6	5.50
Isolate38	Skiff	38	11	6	4.00
Isolate38	Beecher	38	4	6	7.50
Isolate38	Buloke	38	13	6	3.00
Isolate38	CI11458	38	1	6	4.67
Isolate38	Rojo	38	14	6	4.00
Isolate38	Prior	38	8	6	9.50
Isolate38	Corvette	38	9	6	5.50
Isolate22	Vlamingh	22	15	7	2.50
Isolate22	Gilbert	22	12	7	4.50
Isolate22	Harbin	22	7	7	3.00
Isolate22	Kombar	22	10	7	3.00
Isolate22	Prior	22	8	7	10.00
Isolate22	Fleet	22	3	7	5.50
Isolate22	Rojo	22	14	7	3.50
Isolate22	Yerong	22	5	7	8.00
Isolate22	Beecher	22	4	7	3.50
Isolate22	Buloke	22	13	7	3.50
Isolate22	CI11458	22	1	7	4.00
Isolate22	Algerian	22	2	7	5.50

1st leaf, 10

Isolate22	Maritime	22	6	7	4.50
Isolate22	Skiff	22	11	7	4.50
Isolate22	Corvette	22	9	7	9.00
Isolate47	Maritime	47	6	8	4.50
Isolate47	Yerong	47	5	8	3.50
Isolate47	Buloke	47	13	8	2.50
Isolate47	CI11458	47	1	8	2.00
Isolate47	Algerian	47	2	8	3.00
Isolate47	Rojo	47	14	8	1.00
Isolate47	Corvette	47	9	8	6.00
Isolate47	Fleet	47	3	8	4.50
Isolate47	Harbin	47	7	8	2.50
Isolate47	Gilbert	47	12	8	3.50
Isolate47	Skiff	47	11	8	1.50
Isolate47	Prior	47	8	8	4.00
Isolate47	Kombar	47	10	8	5.00
Isolate47	Vlamingh	47	15	8	1.00
Isolate47	Beecher	47	4	8	3.00
Isolate41	Yerong	41	5	9	2.50
Isolate41	Corvette	41	9	9	6.50
Isolate41	Gilbert	41	12	9	3.00
Isolate41	Skiff	41	11	9	4.50
Isolate41	Buloke	41	13	9	4.00
Isolate41	CI11458	41	1	9	3.50
Isolate41	Algerian	41	2	9	4.00
Isolate41	Beecher	41	4	9	4.50
Isolate41	Kombar	41	10	9	4.00
Isolate41	Prior	41	8	9	7.00

Fungal growth

Fungal growth

Isolate41	Harbin	41	7	9	3.00
Isolate41	Maritime	41	6	9	7.00
Isolate41	Fleet	41	3	9	5.00
Isolate41	Rojo	41	14	9	1.00
Isolate41	Vlamingh	41	15	9	2.50
Isolate45	Buloke	45	13	10	1.00
Isolate45	Beecher	45	4	10	5.00
Isolate45	Kombar	45	10	10	2.50
Isolate45	Harbin	45	7	10	1.00
Isolate45	Maritime	45	6	10	5.50
Isolate45	Yerong	45	5	10	3.00
Isolate45	Vlamingh	45	15	10	2.00
Isolate45	CI11458	45	1	10	3.00
Isolate45	Corvette	45	9	10	5.50
Isolate45	Fleet	45	3	10	4.00
Isolate45	Rojo	45	14	10	3.00
Isolate45	Gilbert	45	12	10	5.00
Isolate45	Skiff	45	11	10	2.50
Isolate45	Prior	45	8	10	7.00
Isolate45	Algerian	45	2	10	3.00
Isolate55	CI11458	55	1	11	1.50
Isolate55	Maritime	55	6	11	5.00
Isolate55	Yerong	55	5	11	4.00
Isolate55	Prior	55	8	11	5.00
Isolate55	Algerian	55	2	11	2.00
Isolate55	Kombar	55	10	11	3.00
Isolate55	Harbin	55	7	11	4.00
Isolate55	Fleet	55	3	11	4.00

Fungal growth

Isolate55	Rojo	55	14	11	2.00
Isolate55	Skiff	55	11	11	3.00
Isolate55	Corvette	55	9	11	6.00
Isolate55	Buloke	55	13	11	3.00
Isolate55	Gilbert	55	12	11	4.00
Isolate55	Beecher	55	4	11	5.00
Isolate55	Vlamingh	55	15	11	2.00
Isolate07	Kombar	7	10	12	7.00
Isolate07	Harbin	7	7	12	3.50
Isolate07	Gilbert	7	12	12	7.00
Isolate07	Yerong	7	5	12	8.50
Isolate07	Corvette	7	9	12	7.50
Isolate07	Vlamingh	7	15	12	2.00
Isolate07	Prior	7	8	12	7.00
Isolate07	Skiff	7	11	12	4.50
Isolate07	Beecher	7	4	12	5.50
Isolate07	CI11458	7	1	12	6.00
Isolate07	Rojo	7	14	12	3.50
Isolate07	Maritime	7	6	12	7.50
Isolate07	Fleet	7	3	12	5.00
Isolate07	Algerian	7	2	12	6.50
Isolate07	Buloke	7	13	12	3.00
Isolate35	Skiff	35	11	13	1.00
Isolate35	Beecher	35	4	13	4.00
Isolate35	Maritime	35	6	13	3.00
Isolate35	Gilbert	35	12	13	2.33
Isolate35	CI11458	35	1	13	2.50
Isolate35	Rojo	35	14	13	1.00

Isolate35	Yerong	35	5	13	4.50	
Isolate35	Fleet	35	3	13	1.00	
Isolate35	Kombar	35	10	13	5.00	
Isolate35	Vlamingh	35	15	13	1.00	
Isolate35	Prior	35	8	13	3.00	
Isolate35	Corvette	35	9	13	5.50	
Isolate35	Buloke	35	13	13	1.00	
Isolate35	Harbin	35	7	13	4.33	
Isolate35	Algerian	35	2	13	2.67	
Isolate44	Corvette	44	9	14	5.00	
Isolate44	Prior	44	8	14	6.50	
Isolate44	CI11458	44	1	14	6.67	
Isolate44	Yerong	44	5	14	7.50	
Isolate44	Algerian	44	2	14	6.50	
Isolate44	Harbin	44	7	14	3.50	
Isolate44	Buloke	44	13	14	4.00	
Isolate44	Maritime	44	6	14	4.50	
Isolate44	Vlamingh	44	15	14	2.50	
Isolate44	Gilbert	44	12	14	4.33	
Isolate44	Rojo	44	14	14	3.00	!st leaf has fungal growth
Isolate44	Fleet	44	3	14	8.33	
Isolate44	Beecher	44	4	14	2.50	
Isolate44	Skiff	44	11	14	3.00	
Isolate44	Kombar	44	10	14	4.50	
Isolate68	Fleet	68	3	15	3.67	
Isolate68	CI11458	68	1	15	2.00	
Isolate68	Algerian	68	2	15	2.67	

Isolate68	Buloke	68	13	15	1.50	
Isolate68	Corvette	68	9	15	4.00	
Isolate68	Gilbert	68	12	15	4.50	
Isolate68	Kombar	68	10	15	3.00	too young
Isolate68	Skiff	68	11	15	2.50	
Isolate68	Vlamingh	68	15	15	1.50	
Isolate68	Harbin	68	7	15	1.50	
Isolate68	Maritime	68	6	15	4.50	
Isolate68	Beecher	68	4	15	3.00	
Isolate68	Prior	68	8	15	3.00	1st leaf, 6
Isolate68	Rojo	68	14	15	2.00	
Isolate68	Yerong	68	5	15	2.00	
Isolate09	Rojo	9	14	16	4.00	
Isolate09	Kombar	9	10	16	7.50	
Isolate09	Beecher	9	4	16	8.00	Fungal growth
Isolate09	Skiff	9	11	16	3.50	
Isolate09	Vlamingh	9	15	16	2.50	
Isolate09	Harbin	9	7	16	3.50	
Isolate09	Prior	9	8	16	10.00	
Isolate09	Maritime	9	6	16	8.50	
Isolate09	Gilbert	9	12	16	9.00	
Isolate09	Yerong	9	5	16	7.33	
Isolate09	Fleet	9	3	16	9.00	
Isolate09	Corvette	9	9	16	8.00	
Isolate09	Buloke	9	13	16	3.00	
Isolate09	Algerian	9	2	16	6.50	
Isolate09	CI11458	9	1	16	5.00	
Isolate16	CI11458	16	1	17	2.50	

Isolate16	Vlamingh	16	15	17	2.50	
Isolate16	Prior	16	8	17	9.00	Fungal growth
Isolate16	Rojo	16	14	17	2.50	
Isolate16	Gilbert	16	12	17	6.00	
Isolate16	Fleet	16	3	17	7.00	
Isolate16	Harbin	16	7	17	3.50	
Isolate16	Skiff	16	11	17	5.00	
Isolate16	Yerong	16	5	17	7.67	Fungal growth
Isolate16	Corvette	16	9	17	7.50	
Isolate16	Algerian	16	2	17	5.50	
Isolate16	Beecher	16	4	17	8.00	Fungal growth
Isolate16	Kombar	16	10	17	3.50	
Isolate16	Maritime	16	6	17	7.67	
Isolate16	Buloke	16	13	17	2.50	
Isolate05	Maritime	5	6	18	3.00	too young and dry
Isolate05	Harbin	5	7	18	2.67	
Isolate05	Rojo	5	14	18	3.00	
Isolate05	Vlamingh	5	15	18	1.50	
Isolate05	Corvette	5	9	18	5.33	
Isolate05	Algerian	5	2	18	4.00	
Isolate05	Fleet	5	3	18	3.50	
Isolate05	Buloke	5	13	18	3.00	
Isolate05	Prior	5	8	18	6.67	Clear reticulation
Isolate05	CI11458	5	1	18	2.00	
Isolate05	Skiff	5	11	18	2.50	
Isolate05	Kombar	5	10	18	5.50	
Isolate05	Beecher	5	4	18	3.00	
Isolate05	Gilbert	5	12	18	4.00	
Isolate05	Yerong	5	5	18	7.00	
Isolate45	Beecher	45	4	1	4.00	
Isolate45	Maritime	45	6	1	5.50	
Isolate45	CI11458	45	1	1	2.00	
Isolate45	Kombar	45	10	1	4.33	
Isolate45	Skiff	45	11	1	2.00	
Isolate45	Yerong	45	5	1	8.00	
Isolate45	Algerian	45	2	1	2.00	
Isolate45	Gilbert	45	12	1	6.00	
Isolate45	Fleet	45	3	1	2.00	
Isolate45	Prior	45	8	1	6.00	
Isolate45	Vlamingh	45	15	1	2.00	
Isolate45	Buloke	45	13	1	2.50	
Isolate45	Corvette	45	9	1	2.00	
Isolate45	Rojo	45	14	1	2.00	
Isolate45	Harbin	45	7	1	2.00	
NB29	Algerian	88	2	2	3.67	
NB29	Kombar	88	10	2	#DIV/0!	Plants didn't grow
NB29	Skiff	88	11	2	4.00	
NB29	Gilbert	88	12	2	4.33	
NB29	Prior	88	8	2	4.67	
NB29	Harbin	88	7	2	3.33	
NB29	Maritime	88	6	2	9.00	
NB29	Vlamingh	88	15	2	3.00	
NB29	Yerong	88	5	2	7.00	
NB29	Rojo	88	14	2	2.50	

NB29	Fleet	88	3	2	5.00
NB29	Corvette	88	9	2	3.00
NB29	Buloke	88	13	2	2.00
NB29	Beecher	88	4	2	5.00
NB29	CI11458	88	1	2	2.00
NB85	Harbin	89	7	3	6.00
NB85	Beecher	89	4	3	3.50
NB85	CI11458	89	1	3	4.00
NB85	Vlamingh	89	15	3	3.00
NB85	Fleet	89	3	3	6.00
NB85	Skiff	89	11	3	7.33
NB85	Yerong	89	5	3	8.33
NB85	Gilbert	89	12	3	7.67
NB85	Buloke	89	13	3	6.33
NB85	Rojo	89	14	3	3.50
NB85	Maritime	89	6	3	#DIV/0!
NB85	Prior	89	8	3	10.00
NB85	Algerian	89	2	3	7.00
NB85	Corvette	89	9	3	9.33
NB85	Kombar	89	10	3	4.00
Isolate34	Gilbert	34	12	4	4.67
Isolate34	Yerong	34	5	4	5.67
Isolate34	Vlamingh	34	15	4	2.50
Isolate34	Harbin	34	7	4	7.00
Isolate34	Prior	34	8	4	6.33
Isolate34	Rojo	34	14	4	2.33
Isolate34	CI11458	34	1	4	3.50

Plants didn't grow

Isolate34	Corvette	34	9	4	8.00
Isolate34	Algerian	34	2	4	5.00
Isolate34	Buloke	34	13	4	3.00
Isolate34	Beecher	34	4	4	7.00
Isolate34	Kombar	34	10	4	7.00
Isolate34	Skiff	34	11	4	3.33
Isolate34	Fleet	34	3	4	7.33
Isolate34	Maritime	34	6	4	6.33
Isolate14	Vlamingh	14	15	5	3.00
Isolate14	Rojo	14	14	5	2.50
Isolate14	Algerian	14	2	5	4.00
Isolate14	Maritime	14	6	5	9.00
Isolate14	Prior	14	8	5	8.00
Isolate14	Harbin	14	7	5	2.00
Isolate14	Fleet	14	3	5	9.00
Isolate14	Skiff	14	11	5	3.00
Isolate14	Beecher	14	4	5	6.00
Isolate14	Kombar	14	10	5	5.00
Isolate14	Corvette	14	9	5	10.00
Isolate14	Buloke	14	13	5	2.50
Isolate14	CI11458	14	1	5	3.50
Isolate14	Yerong	14	5	5	5.00
Isolate14	Gilbert	14	12	5	5.50
Isolate36	Corvette	36	9	6	3.00
Isolate36	Skiff	36	11	6	2.50
Isolate36	Gilbert	36	12	6	3.00
Isolate36	Yerong	36	5	6	5.00
Isolate36	Kombar	36	10	6	4.33

Isolate36	Fleet	36	3	6	2.67
Isolate36	Algerian	36	2	6	3.67
Isolate36	Rojo	36	14	6	2.50
Isolate36	CI11458	36	1	6	2.50
Isolate36	Beecher	36	4	6	3.50
Isolate36	Vlamingh	36	15	6	4.00
Isolate36	Prior	36	8	6	4.50
Isolate36	Maritime	36	6	6	6.00
Isolate36	Harbin	36	7	6	3.00
Isolate36	Buloke	36	13	6	3.00
Isolate11	Kombar	11	10	7	4.67
Isolate11	Vlamingh	11	15	7	3.67
Isolate11	Gilbert	11	12	7	3.50
Isolate11	Skiff	11	11	7	3.00
Isolate11	Algerian	11	2	7	2.50
Isolate11	Prior	11	8	7	5.00
Isolate11	Harbin	11	7	7	2.50
Isolate11	Yerong	11	5	7	2.50
Isolate11	Corvette	11	9	7	6.00
Isolate11	Buloke	11	13	7	2.00
Isolate11	Maritime	11	6	7	2.00
Isolate11	Rojo	11	14	7	2.00
Isolate11	Beecher	11	4	7	2.00
Isolate11	CI11458	11	1	7	2.00
Isolate11	Fleet	11	3	7	2.00
Isolate08	Rojo	8	14	8	2.00
Isolate08	Yerong	8	5	8	7.00
Isolate08	Beecher	8	4	8	6.00
Isolate08	Vlamingh	8	15	8	2.00
Isolate08	CI11458	8	1	8	8.33
Isolate08	Harbin	8	7	8	7.00
Isolate08	Gilbert	8	12	8	2.00
Isolate08	Buloke	8	13	8	2.00
Isolate08	Kombar	8	10	8	7.00
Isolate08	Prior	8	8	8	6.50
Isolate08	Fleet	8	3	8	3.00
Isolate08	Algerian	8	2	8	3.50
Isolate08	Skiff	8	11	8	3.00
Isolate08	Maritime	8	6	8	6.00
Isolate08	Corvette	8	9	8	3.50
Isolate47	Gilbert	47	12	9	2.50
Isolate47	Harbin	47	7	9	2.50
Isolate47	Fleet	47	3	9	3.00
Isolate47	Corvette	47	9	9	5.67
Isolate47	Buloke	47	13	9	6.50
Isolate47	Vlamingh	47	15	9	3.00
Isolate47	Skiff	47	11	9	2.50
Isolate47	CI11458	47	1	9	4.00
Isolate47	Rojo	47	14	9	1.00
Isolate47	Beecher	47	4	9	2.00
Isolate47	Algerian	47	2	9	2.00
Isolate47	Yerong	47	5	9	#DIV/0!
Isolate47	Prior	47	8	9	#DIV/0!
Isolate47	Kombar	47	10	9	#DIV/0!
Isolate47	Maritime	47	6	9	#DIV/0!

Plants didn't grow

Plants didn't grow

Plants didn't grow

Plants didn't

grow

Isolate68	Buloke	68	13	10	2.00
Isolate68	Corvette	68	9	10	2.00
Isolate68	Vlamingh	68	15	10	2.00
Isolate68	Beecher	68	4	10	2.00
Isolate68	Rojo	68	14	10	2.00
Isolate68	CI11458	68	1	10	2.00
Isolate68	Kombar	68	10	10	2.00
Isolate68	Harbin	68	7	10	2.00
Isolate68	Prior	68	8	10	2.00
Isolate68	Maritime	68	6	10	2.00
Isolate68	Gilbert	68	12	10	2.00
Isolate68	Skiff	68	11	10	2.00
Isolate68	Fleet	68	3	10	2.00
Isolate68	Algerian	68	2	10	2.00
Isolate68	Yerong	68	5	10	2.00
Isolate22	Prior	22	8	11	9.00
Isolate22	Corvette	22	9	11	6.00
Isolate22	Fleet	22	3	11	3.00
Isolate22	Algerian	22	2	11	3.67
Isolate22	Maritime	22	6	11	5.00
Isolate22	Gilbert	22	12	11	4.00
Isolate22	Rojo	22	14	11	2.00
Isolate22	Kombar	22	10	11	4.00
Isolate22	Beecher	22	4	11	3.00
Isolate22	Yerong	22	5	11	6.00
Isolate22	Skiff	22	11	11	4.00
Isolate22	Harbin	22	7	11	2.50
Isolate22	Buloke	22	13	11	2.50
Isolate22	CI11458	22	1	11	3.00
Isolate22	Vlamingh	22	15	11	3.00
Isolate13	Skiff	13	11	12	2.00
Isolate13	Algerian	13	2	12	2.00
Isolate13	Buloke	13	13	12	3.00
Isolate13	Yerong	13	5	12	2.00
Isolate13	CI11458	13	1	12	2.00
Isolate13	Kombar	13	10	12	5.00
Isolate13	Harbin	13	7	12	5.00
Isolate13	Maritime	13	6	12	7.33
Isolate13	Prior	13	8	12	2.00
Isolate13	Vlamingh	13	15	12	2.00
Isolate13	Corvette	13	9	12	2.00
Isolate13	Fleet	13	3	12	2.00
Isolate13	Beecher	13	4	12	3.00
Isolate13	Gilbert	13	12	12	2.00
Isolate13	Rojo	13	14	12	2.00
Isolate40	Maritime	40	6	13	2.00
Isolate40	Fleet	40	3	13	3.00
Isolate40	CI11458	40	1	13	3.00
Isolate40	Beecher	40	4	13	3.00
Isolate40	Rojo	40	14	13	3.00
Isolate40	Gilbert	40	12	13	3.00
Isolate40	Yerong	40	5	13	3.00
Isolate40	Buloke	40	13	13	3.00
Isolate40	Prior	40	8	13	3.00
Isolate40	Skiff	40	11	13	3.00

Isolate40	Harbin	40	7	13	3.00
Isolate40	Algerian	40	2	13	3.00
Isolate40	Vlamingh	40	15	13	3.00
Isolate40	Kombar	40	10	13	3.00
Isolate40	Corvette	40	9	13	3.00
Isolate64	Prior	64	8	14	2.00
Isolate64	Yerong	64	5	14	2.50
Isolate64	Beecher	64	4	14	3.00
Isolate64	Vlamingh	64	15	14	2.00
Isolate64	Corvette	64	9	14	2.00
Isolate64	Buloke	64	13	14	2.00
Isolate64	Kombar	64	10	14	2.00
Isolate64	Harbin	64	7	14	2.00
Isolate64	Maritime	64	6	14	4.00
Isolate64	CI11458	64	1	14	2.00
Isolate64	Rojo	64	14	14	2.00
Isolate64	Gilbert	64	12	14	2.00
Isolate64	Algerian	64	2	14	2.00
Isolate64	Fleet	64	3	14	2.00
Isolate64	Skiff	64	11	14	2.00
Isolate04	CI11458	4	1	15	3.00
Isolate04	Prior	4	8	15	2.00
Isolate04	Corvette	4	9	15	2.00
Isolate04	Fleet	4	3	15	2.00
Isolate04	Buloke	4	13	15	3.00
Isolate04	Rojo	4	14	15	3.00
Isolate04	Vlamingh	4	15	15	3.00
Isolate04	Algerian	4	2	15	3.00

Isolate04	Maritime	4	6	15	3.00
Isolate04	Harbin	4	7	15	3.00
Isolate04	Beecher	4	4	15	3.00
Isolate04	Skiff	4	11	15	3.00
Isolate04	Gilbert	4	12	15	3.00
Isolate04	Yerong	4	5	15	3.00
Isolate04	Kombar	4	10	15	3.00
Isolate62	Fleet	62	3	16	5.33
Isolate62	Buloke	62	13	16	5.33
Isolate62	Algerian	62	2	16	5.67
Isolate62	Corvette	62	9	16	7.67
Isolate62	Maritime	62	6	16	6.67
Isolate62	Beecher	62	4	16	7.00
Isolate62	CI11458	62	1	16	3.00
Isolate62	Skiff	62	11	16	3.33
Isolate62	Prior	62	8	16	7.67
Isolate62	Kombar	62	10	16	5.67
Isolate62	Yerong	62	5	16	6.67
Isolate62	Gilbert	62	12	16	4.67
Isolate62	Harbin	62	7	16	3.33
Isolate62	Rojo	62	14	16	3.67
Isolate62	Vlamingh	62	15	16	2.00
Isolate30	Beecher	30	4	17	3.50
Isolate30	Maritime	30	6	17	7.00
Isolate30	CI11458	30	1	17	2.50
Isolate30	Kombar	30	10	17	5.50
Isolate30	Skiff	30	11	17	4.67
Isolate30	Yerong	30	5	17	5.33

Isolate30	Algerian	30	2	17	6.00
Isolate30	Gilbert	30	12	17	4.00
Isolate30	Fleet	30	3	17	3.00
Isolate30	Prior	30	8	17	8.00
Isolate30	Vlamingh	30	15	17	2.50
Isolate30	Buloke	30	13	17	3.00
Isolate30	Corvette	30	9	17	5.00
Isolate30	Rojo	30	14	17	3.00
Isolate30	Harbin	30	7	17	3.00