INDUSTRY BIOSECURITY PLAN
FOR THE GRAINS INDUSTRY

Threat Specific Contingency Plan

Net form of net blotch (exotic pathotypes)

Pyrenophora teres f. sp. *teres*

Prepared by Trevor Bretag

and Plant Health Australia

May 2009

Plant Health AUSTRALIA

GRDC Grains Research & Development Corporation

CRC PLANT biosecurity

Disclaimer

The scientific and technical content of this document is current to the date published and all efforts were made to obtain relevant and published information on the pest. New information will be included as it becomes available, or when the document is reviewed. The material contained in this publication is produced for general information only. It is not intended as professional advice on any particular matter. No person should act or fail to act on the basis of any material contained in this publication without first obtaining specific, independent professional advice. Plant Health Australia and all persons acting for Plant Health Australia in preparing this publication, expressly disclaim all and any liability to any persons in respect of anything done by any such person in reliance, whether in whole or in part, on this publication. The views expressed in this publication are not necessarily those of Plant Health Australia.

Further information

For further information regarding this contingency plan, contact Plant Health Australia through the details below.

Address:	Suite 5, FECCA House 4 Phipps Close DEAKIN ACT 2600
Phone:	+61 2 6260 4322
Fax:	+61 2 6260 4321
Email:	**admin@phau.com.au**
Website:	**www.planthealthaustralia.com.au**

1 Purpose of this Contingency Plan

This Contingency Plan provides background information on the pest biology and available control measures to assist with preparedness for an incursion into Australia of new pathotypes of the Net form of net blotch (*Pyrenophora teres* f. sp. *teres*). It provides guidelines for steps to be undertaken and considered when developing a Response Plan for new pathotypes of Net form of net blotch. Any Response Plan developed using information in whole or in part from this Contingency Plan must follow procedures as set out in PLANTPLAN (Plant Health Australia, 2008) and be endorsed by the National Management Group prior to implementation.

2 Pest information/status

2.1 Pest details

Pyrenophora teres f. sp. *teres*

Other Scientific Names: *Drechslera teres* f. sp. *teres*

Common Names: Net form of net blotch, NFNB

2.1.1 General information

Taxonomic position – Class: Ascomycetes; Subclass: Dothideomycetidae; Order: Pleosporales; Family: Pleosporaceae

Net blotch is a destructive foliar fungal disease of barley, caused by the *Pyrenophora teres* fungus, with infection reducing both yield and quality of the grain (Hims, 1987). The disease occurs in two forms – net form of net blotch (NFNB; *P. teres* f. sp. *teres*) and spot form of net blotch (SFNB; *P. teres* f. sp. *maculata*) (Hollaway & McLean, 2008). Both forms of the disease result in lesions forming on the leaves, or occasionally on the sheaths. The net form symptoms start as pinpoint brown lesions on the leaves which elongate and produce fine, dark brown streaks along and across the leaf blades. This spread creates the distinctive net-like pattern. As the disease continues to develop the lesions continue to elongate, following the veins, and are often surrounded by a yellow margin. The spot form also starts as small lesions, but these do not elongate, and become surrounded by a chlorotic zone of varying widths. Spot lesions can grow up to 3-6 mm in diameter. Different lesion types can occur, depending on the barley genotype and the strain of the pathogen (Steffenson, 1997).

The main infection sources are spores that over-winter on seeds, crop residues or stubble (Jordan & Allen, 1984). High infection rates are generally seen when barley is planted in the same field as infected barley crops from the year before, particularly if the stubble was not deeply buried (Turkington *et al.,* 2006). Airborne spore transmission of the pathogen occurs over short distances (generally within 7 metres of the source), and is positively related to temperature (Martin *et al.,* 1984). Infection rates are dependent on humidity, with most infections requiring between 10 and 30 hours of high humidity. The optimal temperature for sporulation is between 10 and 30°C (Sato & Takeda, 1990). The disease cycle can be completed in less than 14 days under ideal conditions (Shaw, 1986).

A large number of *P. teres* f. sp. *teres* pathotypes have been identified from a number of countries (e.g. 45 from Canada (Tekauz, 1990) and two from Australia (Serenius, 2006; Serenius *et al.*, 2007).

While there is no universally accepted barley line used to test the virulence of the different pathotypes, high pathotype diversity exists within *P. teres* (Tekauz & Buchannon, 1977; Steffenson & Webster, 1992; Robinson & Mattila, 2000). Inheritance of virulence in the pathogen is consistent with this trait being under the control of a single, major gene (Weiland *et al.,* 1999). Cultivars of barley vary significantly in their resistance to the pathogen, ranging from susceptible through to highly resistant (Harrabi & Kamel, 1990; Robinson & Jalli, 1996; Douiyssi *et al.,* 1998; Hollaway 2009). Host susceptibility is normally highest during the seedling stage, with mature plants showing higher levels of resistance. Therefore, reducing infections by removing diseased plant residues from past seasons is important in minimising crop damage. The most effective means of controlling net blotch is the use of resistant cultivars (Steffenson, 1997).

In Australia, the incidence and severity of NFNB is low on most cultivars but can reach damaging levels on susceptible cultivars. Any report of serious disease outbreaks in resistant cultivars should be investigated immediately to establish whether there has been a change in the virulence of the pathogen or an incursion of a more virulent strain of *P. teres* f. sp. *teres*. Incursions of new strains may also be suspected if a seed treatment with fungicides fails to effectively control seed-borne infection.

2.1.2 Life cycle

P. teres f. sp. *teres* inoculum survives from one season to the next as seed-borne mycelium or as mycelium and pseudothecia on infested host residues. The relative importance of the different inoculum sources can vary depending on growing conditions (van den Berg, 1988; McLean *et al.,* 2009).

Although seed harvested from barley crops affected by net blotch is likely to be infected with *P. teres* f. sp. *teres* (Jordan, 1981) and can be important in initiating net blotch epidemics, the main importance of seed-borne inoculum is the introduction of the pathogen into fields previously free of the disease (Steffenson, 1997). Infection of barley seedlings by seed-borne inoculum is greatest at low temperatures (10-15°C) and rarely occurs above 20°C. Infection quickly spreads to the coleoptile and emerging leaves. Conidia formed from the lesions on the infected leaves can serve as secondary inoculum and spread the pathogen to adjacent plants. Symptoms arising from seed transmission are usually distinguishable from those resulting from air- or splash-dispersed conidia or from ascospores.

In Australia, most barley seed is treated with a fungicide, which reduces the likelihood of seed to seedling infection.

In most barley growing regions, conidia and ascospores formed on infected crop residues are the main sources of primary inoculum (Piening, 1968). Infected volunteer barley or alternative hosts, which survive between cropping seasons, can also serve as sources of primary inoculum for newly sown crops.

The fungus can survive for up to 2 years as mycelium and pseudothecia on infected barley stubble. Ascospores and conidia are dispersed by wind and rain and infect volunteer plants and newly emerging barley seedlings. Ascospores are forcibly discharged and may be carried large distances, however, conidia are not forcibly released and most travel less than 7 m from the source (Steffenson, 1997). Sporulation occurs when the relative humidity is near 100% and temperatures range from 15-25°C.

The optimum temperature for infection of barley leaves is also 15-25°C, but infection can occur from 8-30°C. Infection may occur after a moist period of 5 h, but is greatest when humid conditions persist for 10-30 h. Infection times are increased under drier conditions and at temperatures above 25°C disease development can be halted (Sato & Takeda, 1990; van den Berg & Rossnagel, 1990). Under

optimal conditions net blotch lesions develop and sporulation occurs within 10 days of initial infection and can kill a leaf within 15 days (van den Berg, 1988).

Conidia produced on the surface of primary lesions serve as secondary inoculum.

The development of net blotch epidemics is dependent on the amount of primary inoculum, the number of cycles of secondary infection, and host susceptibility. The disease cycle can be completed in 10 to 14 days under optimal conditions (Shipton *et al.*, 1973).

2.2 Affected hosts

2.2.1 Host range

P. teres f. sp. *teres* shows a strong preference for barley (*Hordeum vulgare*) plants, but will also infect oats (*Avena sativa*), wheat (*Triticum aestivum*) and other *Hordeum* spp. (Shipton *et al.*, 1973). It is also known to infect the wild species awnless brome (*Bromus inermis*) and a number of *Poaceae* spp.

In California, 95 grass species were screened in the field for their reactions to *P. teres* f. sp. *teres* and 65 species developed disease symptoms (Brown *et al.*, 1993). The net blotch fungus was reisolated from all species infected in the field trial, and these isolates retained their pathogenicity on barley after a single passage through alternative hosts. The results showed that 15 of the 16 grass genera tested have susceptible species.

The broad host range of *P. teres* f. sp. *teres* under field conditions indicates the potential of alternative hosts as sources of primary inoculum.

2.2.2 Geographic distribution

Steffenson (1997) reported that net blotch of barley occurred in most barley-growing regions of the world, but was most severe in temperate regions of high rainfall and humidity.

P. teres f. sp. sp. *teres* is present in countries of Europe, Asia, Africa, Americas and Oceania, as follows:

- Asia: Afghanistan, Armenia, China, India, Iran, Iraq, Israel, Japan, Republic of Korea, Kyrgyzstan, Myanmar, Nepal, Pakistan, Turkey, Turkmenistan, Uzbekistan
- Europe: Austria, Baltic States, Bulgaria, Cyprus, Denmark, Faroe Islands, Finland, Former USSR, Former Yugoslavia, France, Germany, Greece, Ireland, Italy, Malta, Moldova, Netherlands, Norway, Poland, Romania, Russia, Spain, Sweden United Kingdom
- Africa: Egypt, Ethiopia, Kenya, Libya, Morocco, Saint Helena, South Africa, Tanzania, Tunisia, Zambia
- North America: Canada, Mexico, USA
- South America: Argentina, Brazil, Colombia, Peru, Uruguay
- Oceania: Australia, New Zealand

2.2.3 Symptoms

Net blotch is named for the netlike symptoms commonly produced on barley leaves. Hollaway & McLean (2008) provide excellent descriptions of the symptoms and images of the net blotches of barley.

NFNB symptoms occur mainly on the leaves of barley plants, with minor symptoms present on sheaths, seeds and inflorescence. Infection occurs more rapidly in fresh, healthy leaves compared with older, drier leaves (Shaw, 1986). Small circular to elliptical brown spots appear on leaves and enlarge into, narrow, dark brown and transverse striations, forming the characteristic netlike pattern. The affected part of the leaf turns brown and the adjoining tissues become chlorotic and areas of dead tissue can form. The lesions are usually restricted in width by the leaf veins in adult plants but can extend up to 25 mm in length. Severely infected leaves may become completely necrotic and dry up (Shipton *et al.*, 1973).

Small brown streaks develop on the glumes, while infected seed have indistinct brown lesions on their bases. The fungus produces pseudothecia on infected tissues when the plant has matured.

2.3 Entry, establishment and spread

When the Industry Biosecurity Plan for the Grains Industry was prepared in 2004 and more recently reviewed in 2009, exotic pathotypes of *P. teres* f. sp. *teres* were given an overall risk rating of High. This contingency plan has been commissioned noting the implication to the grains industry for the entry of exotic pathotypes with possible fungicide resistance, also acknowledging that certain *P. teres* f. sp. *teres* pathotypes are already present and management practices are currently used.

2.3.1 Entry potential

Rating: High

The entry potential of new pathotypes of *P. teres* f. sp. *teres* is high for the following reasons:

- *P. teres* f. sp. *teres* pathotypes have already entered the country and become established on barley and alternative plant species in most barley growing regions within Australia. The pathogen is currently being managed and maintained
- *P. teres* f. sp. *teres* can be seed-borne and survive on infected plant residues for several years
- The risk would be reduced if more stringent conditions were imposed on the importation of barley seed

2.3.2 Establishment potential

Rating: High

The establishment potential of new pathotypes of *P. teres* f. sp. *teres* in Australia is high for the following reasons:

- *P. teres* f. sp. *teres* already occurs in Australia on barley, demonstrating that suitable conditions do occur in Australia for the pathogen to survive

- Climatic conditions between countries where the disease already occurs and areas of Australia are similar
- Current barley cultivars in Australia vary in their susceptibility to NFNB. Cultivars range from moderately resistant to highly susceptible. In South Australia, high levels of NFNB have been observed in some crops of the barley cultivar Keel
- Pathotypes of *P. teres* f. sp. *teres* already in Australia can be effectively managed through the use of cultivar selection, crop rotation and fungicides, however the possible management of new pathotypes would need to be determined
- Fungicide resistances in *P. teres* f. sp. *teres* to prochloraz (Serenius & Manninen, 2006) and triadimenol (Sheridan *et al.*, 1985; Campbell & Crous, 2002) have been reported. It is unknown whether new pathotypes of NFNB will have fungicide resistance.

2.3.3 Spread potential

Rating: High

The spread potential of new pathotypes of *P. teres* f. sp. *teres* in Australia is considered high for the following reasons:

- Destruction of host residue infested with *P. teres* f. sp. *teres* is often difficult because the pathogen can survive on debris for at least 2 growing seasons
- Ascospores of *P. teres* f. sp. *teres* can be wind-borne over large distances
- The fungal spores can be splash dispersed over short distances
- Moving water can carry infected crop residues short distances to surrounding plants and adjoining paddocks
- The pathogen can be transported over large distances in infected grain and harvesting equipment into new areas
- Windblown plant debris could spread the pathogen over moderate distances following harvest into adjacent paddocks

2.3.4 Economic impact

Rating: Medium

The impact of new pathotypes of *P. teres* f. sp. *teres* in Australia is medium for the following reasons:

- Current cultivars vary in their susceptibility to NFNB, but their susceptibility to new pathovars is unknown
- NFNB can reduce grain yields in susceptible cultivars by more than 50%
- Yield losses are likely to be less than 10% if resistant cultivars are available
- High risk of a NFNB epidemic developing if barley is planted in or near a previous barley crop
- Susceptible barley plants cannot be grown in infected fields in the following season, unless plant residue was deep ploughed, resulting in the reduction in the possible field output

2.3.5 Environmental impact

Rating: Very Low

There is no potential to degrade the environment or otherwise alter the ecosystem by affecting species composition or reducing the longevity or competitiveness of wild hosts.

Increased use of fungicides used to control NFNB may have a detrimental effect on the environment.

2.3.6 Overall risk

Rating: Medium

2.4 Diagnostic information

2.4.1 Diagnostic protocol

Reliable techniques are required to detect *P. teres* f. sp. *teres* on seed, growing plants and plant debris. In addition, the fungal isolates detected need to be tested for their pathogenicity and sensitivity to fungicides.

The main concern with the introduction of new strains of *P. teres* f. sp. *teres* is that they may be more virulent than local strains of the fungus or have resistance to fungicides currently being used to control NFNB.

P. teres f. sp. *teres*, the causal agent of NFNB and *P. teres* f. sp. *maculata*, the causal agent of SFNB, are morphologically identical and can only be separated based on symptoms and by molecular characterisation (Williams *et al.*, 2001).

On plants taken from the field, NFNB and SFNB can usually be identified by the leaf symptoms they produce. However, molecular techniques are required to confirm their identity. In recent years, several techniques have been developed to enable the positive identification of *P. teres* f. sp. *teres*.

Williams *et al.* (2001) developed a test based on the polymerase chain reaction to distinguish between NFNB and SFNB formae. This assay can now be used routinely for the identification of each pathogen from infected plant tissues.

Leisova *et al.* (2005) developed specific polymerase chain reaction (PCR) primers from amplified fragment length polymorphism (AFLP) fragments of *P. teres*. The primers were designed specifically to amplify DNA from *P. teres* f. sp. *teres* (net form) and allow its differentiation from *P. teres* f. sp. *maculata* (spot form). No cross-reaction was observed with DNA of several other species, such as *P. tritici-repentis*, *P. graminea* and *Helminthosporium sativum*.

Leisova *et al.* (2006) also developed a diagnostic system based on real-time PCR and used it to quantify the occurrence of both forms of *P. teres* in infected barley leaves during the growing season. TaqMan MGB (Minor Groove Binder) primers and probes were designed that showed high specificity for each of the two forms of *P. teres*.

A real-time PCR based assay that can determine the level of infection in seeds has also been developed (Bates & Taylor, 2001). This technique utilises the Scorpion Amplified Refractory Mutation System (ARMS), and can determine the level of infection as well as distinguishing between *P. teres* and *P. graminea* infection in a single reaction. This approach improved on the standard PCR-based assay (Thomas *et al.,* 1998), which required two reactions to determine which species of pathogen is

present. The real-time PCR based assay also provides higher levels of sensitivity and quantitation than the standard PCR assay.

Blotter or agar plate tests can be used to detect *P. teres* on seed. The identity of the fungus is confirmed by microscopic examination of seed and fungal colonies, based on descriptions by Ellis & Waller (1973). However, molecular techniques are still required to distinguish between NFNB and SFNB formae.

Three common methods for detecting *P. teres* on seed are listed below.

- Freezer blotter test (Joergensen, 1977):
    - Surface sterilize seeds in 1% sodium hypochlorite solution for 10 min
    - Place seeds on moist blotters and incubate for 24 h at 20°C followed by 24 h at -20°C
    - Incubate at 20°C for 5 d under 12 h of darkness and 12 h of near-ultraviolet (NUV) light
    - Examine the seeds under the microscope for conidia of *P. teres*
- Blotter test (Hampton & Matthews, 1980):
    - Surface sterilize seeds in 1% sodium hypochlorite solution for 10 min
    - Incubate seeds for 7 d at 20°C under a 10 h NUV light and 14 h dark cycle
    - Examine seeds for the presence of conidia or conidiophores of *P. teres*
- Malt agar (Hampton & Matthews, 1980):
    - Surface sterilize seeds in 1% sodium hypochlorite solution for 10 min
    - Incubate seeds on 2% malt agar for 5 d at 20°C in the dark
    - Incubate for 2 d at 20°C under a 10 h NUV light and 14 h dark cycle
    - Examine plates for colonies of *P. teres*

These techniques can also be used to isolate the fungus from infected crop debris.

Keiper *et al*. (2008) reported that they had also developed diagnostic micro satellite markers for the barley net blotch pathogens, *P. teres* f. sp. *teres* and *P. teres* f. sp. *maculata* which may be useful.

Laboratory and/or glasshouse testing is required to determine the sensitivity of the isolates to the fungicides currently used in Australia to control NFNB. Campbell & Crous (2002) outlined methods for determining the sensitivity of *P. teres* f. sp. *teres* to different fungicides.

The pathotypes of each fungal isolates can be determined by inoculating a differential set of barley cultivars. Jonsson *et al.* (1997) developed a differential set of barley lines that can be used in assessing pathogen virulence in *P. teres* f. sp. *teres*.

2.5 Response checklist

Guidelines for Response Checklists are still to be endorsed. The following checklist provides a summary of generic requirements to be identified and implemented within a Response Plan:

- Destruction methods for plant material, soil and disposable items
- Disposal procedures
- Quarantine restrictions and movement controls

- Decontamination and farm cleanup procedures
- Diagnostic protocols and laboratories
- Trace back and trace forward procedures
- Protocols for delimiting, intensive and ongoing surveillance
- Zoning
- Reporting and communication strategy

Additional information is provided by Merriman & McKirdy (2005) in the Technical Guidelines for Development of Pest Specific Response Plans.

2.6 Delimiting survey and epidemiology study

Testing of barley plants for the presence of *P. teres* f. sp. *teres* should only occur when resistant cultivars show symptoms of NFNB or registered fungicides fail to adequately control the disease.

Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas of diseased plants. The normal procedure is to collect symptomatic plants and to test them to confirm the presence of *P. teres* f. sp. *teres*, however identification of new pathotypes may require assessment of cultivar or chemical susceptibility. If confirmed, plants taken at random from the same crop should be tested to enable an estimate to be made of the disease incidence. Surrounding crops would then be surveyed. The extent of the survey beyond the initial infected crop should be guided by the test results from surrounding crops.

Seed trace-back will indicate how many seed lots and crops will need to be tested. If the seed used has been sown at several sites, delimiting surveys should be conducted at each site.

2.6.1 Sampling method

Once initial samples have been received and preliminary diagnosis made, follow up samples to confirm identification of the pathogen will be necessary. This will involve sampling directly from the infected crop, and sampling crops over a larger area to determine the extent of disease distribution.

From each crop sampled, at least 100 plants should be taken at random. However, preference may be given to symptomatic plants in fields where the disease incidence is low.

All plants should be assessed for the presence of the NFNB symptoms (i.e. light brown lesions containing thin mesh/nets of darker brown). Lesions on NFNB infected plants start out small and elongate, with more advanced infections elongating as a strip along the leaf and be surrounded by a chlorotic, yellow zone.

Any personnel collecting samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis. General protocols for collecting and dispatching samples are available within Appendix 3 of PLANTPLAN (Plant Health Australia, 2008).

The total number of samples collected at this point may run into the hundreds or even thousands. It is vital that a system of sample identification is determined early in the procedure to allow for rapid sample processing and accurate recording of results. Follow up samples will be forwarded to the nominated diagnostic laboratories for processing.

Samples should be initially collected over a representative area of the infected crop to determine the pathogen distribution. The disease may appear as patches within the crop depending on the source of the pathogen.

Depending on the stage of infection the symptoms may appear as:

- Plants with small circular to elliptical light brown spots
- Plants with light brown lesions containing thin mesh/nets of darker brown
- Patches of plants with severe leaf damage within the crop

It is important to note the distribution of disease in the initial crop, as this will indicate whether the pathogen has been seed-borne, carried on trash from adjacent paddocks or originated from contaminated machinery or human movement.

It is vitally important that all personnel involved in crop sampling and inspections take all precautions to minimise the risk of disease spread between crops by decontaminating between paddocks.

Samples should be collected from plants that represent a range of symptoms observed in the infected crop. Preferably enough material should be collected to allow for immediate processing and retention of a portion that can be placed into long term storage as a reference.

Samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state. An esky with ice packs or portable fridge should be carried when sampling crops. Samples should be wrapped in damp newspaper, bundled into a plastic bag and clearly labelled. For appropriate labelling and packaging procedures for suspect emergency plant pests consult PLANTPLAN (Plant Health Australia, 2008).

Samples should be processed as quickly as possible after sampling from the field if sub cultures are to be made from infected tissue. Once removed from the field, fresh plant samples can deteriorate and become contaminated by other mould fungi and bacteria, which may prevent successful sub-culturing of the pathogen. Sub-culturing should be done within three to four days after sampling from the field. Infected plant tissue to be used for PCR analysis can be placed in a -80^{o}C freezer and stored for an indefinite period without damaging fungal DNA.

Long term storage of isolates can occur as fungal cultures can be freeze dried for future reference (without loss of viability) or as deep frozen plant specimens maintained at -80^{o}C, which can be used to extract DNA.

It is important to record the precise location of all samples collected, preferably using GPS, or if this is not available, map references including longitude and latitude and road names should be recorded. Property and owners names should also be included where possible.

It is important that all diagnoses of suspected exotic pathotypes are undertaken according to the following parameters:

- The laboratory diagnostician has expertise in this form of diagnosis
- The test is undertaken as described by Williams *et al.* (2001)
- The results are confirmed by diagnosis in another recognised laboratory or by another diagnostician
- Where possible diagnosis is confirmed by a second method

2.6.2 Epidemiological study

The number of infected plants within a crop will depend on the amount of inoculum available and whether conditions have been favourable for the pathogen to spread from the initial foci.

Sampling of crops within a district and beyond will be based upon the origins of the initial suspect sample(s). Factors to consider will be:

- The source of seed used and how long that seed has been used by the grower
- If any other barley crops have been sown from the same source seed
- The proximity of other barley crops to the infected crop, both in the current growing season and previous season. This will include the growers own crops and crops on neighbouring properties
- What machinery or vehicles have been into the infected crop?
- The extent of human movements into the infected crop. A possible link to recent overseas travel or visitors from other regions should also be considered.

2.6.3 Models of spread potential

No modelling data are available.

Spread may occur in the following ways:

- Movement of infected seed. The pathogen has the potential to be transmitted as infected seed. Seed to seedling transmission has been documented and this pathway of dispersal should not be ignored. Small infected fragments can also be carried within infested seed lots.
- Mechanical transmission through movement on contaminated vehicles and machinery
- Small fragments of stem pieces carrying fungal mycelium and pseudothecia can be blown into surrounding paddocks during harvesting and allow the pathogen to move considerable distances away from the infected crop
- Ascospores released from pseudothecia on infected crop residues may be carried large distances into new crops
- Fungal spores can be dispersed before a crop reaches maturity and result in secondary spread of the disease. Within a crop the spores are usually dispersed relatively short distances by rain splash but can sometimes be carried to neighbouring crops by windblown rain
- Fungal spores that adhere to clothing, machinery or animals can be carried large distances into other barley crops

2.6.4 Pest Free Area guidelines

The establishment and maintenance of pest free areas (PFAs) can be a resource-intensive process, especially as pathotypes of NFNB already occur within Australia. Prior to development of a PFA due consideration should be given to alternative methods (e.g. treatments or enclosed quarantine) that achieve an equivalent biosecurity outcome to a PFA. A benefit-cost analysis is useful for this purpose.

Additional information is provided by the IPPC (1995) in Requirements for the Establishment of Pest Free Areas. This standard describes the requirements for the establishment and use of PFAs as a risk management option for phytosanitary certification of plants and plant products. Establishment of maintenance of a PFA can vary according the biology of the pest, pest survival potential, means of dispersal, availability of host plants, restrictions on movement of produce, as well as PFA characteristics (size, degree of isolation and ecological conditions).

Points to consider are:

- Design of a statistical delimiting field survey for symptoms on host plants (See 2.6.1 for points to consider in the design)
- Plant sampling should be based on at least 100 plants taken at random per crop
- Preliminary diagnosis can be based on leaf symptoms and fungal morphology
- PCR methods are required to distinguish between NFNB and SFNB
- Seed sampling should be based on a minimum of 400 seeds (preferably 1000) as infection levels in seed can be low
- Surveys should also consider alternative host plants, in particular other cereals and barley grass
- The use of aerial inspection or remote sensing may be possible, with suspect patches inspected and sampled to confirm or deny the presence of NFNB

2.7 Availability of control methods

2.7.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms
- Ensure that seed production does not take place on affected farms and do not use seed from these farms to plant new crops as NFNB can be seed borne
- After surveys are completed, destruction of infected crops and seed lots should be undertaken. Infected crops should be destroyed by burning and ploughing. Any infected seed lots should be incinerated or buried deeply (in a non-cropping area)
- On-going surveillance of infected paddocks to ensure NFNB is eradicated

2.7.2 Control if small areas are affected

As above.

2.7.3 Control if large areas are affected

A large area may become affected if a large quantity of infected seed has been widely distributed or if the disease has gone unnoticed for a number of years.

Implementation of large area controls will depend on the ability to determine the original source and track/trace the spread. It will also depend on whether the source is infected barley seed or another source (e.g. contaminated clothing or machinery). If the pathogen is found to be confined to a single seed lot and only found in barley crops, it may be possible to eradicate the pathogen by destroying all barley and other susceptible crops in that region. Infected crops should be destroyed by burning and ploughing. However, unless the pathogen is detected very early it is unlikely that disease eradication will be possible. The fungus is able to survive on crop residues for several years and be spread by vehicles and farm machinery. Infected plant debris are also likely to be spread by wind over moderate distances.

Because the fungus can survive for several years, a break of at least three years (preferably longer) would be needed before barley could be safely grown again in the same area. Non-susceptible crops such as pulses and oilseeds may be planted during this period.

All equipment used on the site should be thoroughly cleaned down, with products such as a farm degreaser or a bleach (1% available chlorine) solution and washed down with a pressure cleaner on the affected farm. The clean down procedure should be carried out on hard standing or preferably a designated wash-down area to avoid mud being recollected from the affected site onto the machine. A wash-down pad design and operational procedures are described in the cotton IDM package.

Where eradication is attempted, there needs to be ongoing monitoring of infected paddocks to ensure there is no opportunity for the pathogen to re-establish on self sown plants.

If eradication or containment is not possible, control measures need to be implemented to minimise the impact of the disease. Destruction of primary inoculum is the first step towards controlling NFNB. The use of pathogen-free seed and/or fungicide treated seed will eliminate seed-borne inoculum and prevent the introduction of *P. teres* f. sp. *teres* into areas free of the pathogen. Destruction of crop residues infected with *P. teres* f. sp. *teres* by burning or deep-ploughing is also recommended. Rotation with non-susceptible crops for at least 3 years will also reduce the likelihood of disease carry-over. Only cultivars resistant to NFNB should be planted. Foliar fungicides may also be used to control the disease during the growing season.

2.7.4 Cultural control

Cultural control may be possible by growing non-host crops such as pulses and oilseeds as this would enable ongoing spraying with selective herbicides of any grasses or self-sown cereals. This would remove any potential hosts of the pathogen.

NFNB infections can be significantly reduced through employing effective cultural controls. The major source of over-wintering inoculum for infection is from crop stubble and other plant debris, particularly where high infection rates were present in the previous session. The pathogen can survive on crop debris for over two years. Removal of all crop stubble at the end of the season, through deep ploughing, appreciably reduces inoculum source contamination (Jordan & Allen, 1984). High tillage farming systems should be employed to limit net blotch incidence as minimum tillage farming practices can increase the disease incidence and cause yield losses of over 30% (Platz *et al.,* 2001).

Crop rotation is another effective procedure for reducing NFNB infection rates and effects on crops. Follow a crop rotation for at least 3 years with non-susceptible hosts. If there is a requirement to grow two successive barley crops, then only grow resistant cultivars.

NFNB can be seed borne if infection of plants occurred before maturity. Therefore, ensuring seeds were produced from pathogen-free plants is essential to reducing NFNB incidence. Seed infection levels can also be tested through blotting/growing or PCR-based methods. Such analysis can determine whether seed is free from the pathogen or the overall infection rates.

The use of heavy nitrogen applications generally produces conditions more favourable for outbreaks of the disease. Therefore, use balanced applications of nitrogen and phosphorus fertilisers.

2.7.5 Host plant resistance

The use of resistant lines is an important part of an effective disease management program for NFNB. Cultivars of barley currently used throughout the world vary significantly in their resistance to the pathogen, ranging from highly resistant to highly susceptible (Harrabi & Kamel, 1990; Robinson & Jalli, 1996; Sheridan, 1997; Douiyssi *et al.,* 1998). Australian barley cultivars range from moderately resistant to very susceptible to NFNB (Anonymous, 2008). Consult a current Cereal Disease Guide (available on the VIC DPI website **www.dpi.vic.gov.au**) when selecting resistant cultivars. However, resistant cultivars will need to be reviewed following an incursion of a new pathotype of NFNB.

The barley cultivar Keel, which was considered moderately resistant to NFNB when first evaluated, has been severely affected by the disease in recent years. This suggests that more virulent strains of the fungus may have evolved or been introduced into Australia. There are reports of considerable pathogenic variability between isolates of *P. teres* f. sp. *teres* (Steffenson & Webster, 1992; Wu *et al.* 2003). For durable resistance, cultivars may need to have multiple resistance genes.

Some cultivars have strong resistance to SFNB but only moderate resistance to NFNB (e.g. Helm *et al.,* 2004). A number of dominant resistance genes have been identified in barley cultivars – two from Australia (Khan & Boyd, 1969), two from Sweden (Jonsson *et al.,* 1999), and one each from Canada and Egypt (Buchannon & McDonald, 1965). Development of resistant lines of barley to net blotch through molecular marker assisted breeding programs is currently underway in Australia. A number of markers for resistance have been identified in barley, with one region on chromosome 6H producing highly significant association with a resistant phenotype (Cakir *et al.,* 2003).

Testing of 109 barley lines against 14 pathotypes of NFNB showed only one line to be resistant to all pathotypes (Jonsson *et al.,* 1997), suggesting that the use of resistant plants in combating NFNB infection must be completed with prudence.

2.7.6 Chemical control

Fungicide applications should only be used as an adjunct to cultural control and host-plant resistance measures outlined above.

In Australia, Dividend® (difenoconazole plus metalaxyl-M) is the only seed treatment registered for the control of seed-borne net blotch in barley.

Carboxin plus thiram has also been reported to be an effective seed treatment against net blotch in barley (Martin, 1985). Triadimenol seed treatment was very effective against net blotch when first used, however, resistance to the fungicide developed very quickly (Sheridan *et al*., 1985, Cadorin & Reis, 2003).

The following foliar fungicides have been reported to be effective in Australia against NFNB:

- Propiconazole (Registered in Australia for the control of net blotch)
- Azoxystrobin
- Cyproconazole
- Epoxiconazole

Jordan and Best (1984) reported that prochloraz was the most effective chemical they tested against net blotch, however, some *P. teres* f. sp. *teres* populations have now become tolerant to the fungicide (Serenius & Manninen, 2006).

2.7.7 Mechanical control

Burying and burning of infected barley stubble significantly reduces the amount of air-borne inoculum available and reduces the amount of disease (Jordan & Allen, 1984).

2.7.8 Biological control

The bacterial bio-control agent *Pseudomonas chlororaphis,* strain MA 342, was tested for activity against *P. teres* in Sweden during 1991-1996 (Johnsson *et al*., 1998). A bacterial liquid culture was directly applied to pathogen-infected seeds of barley without additives. The seeds were then dried and sown in the field together with fungicide-treated and untreated seeds used as controls. The bacterization controlled *P. teres* as effective as guazatine + imazalil, and these effects were consistent over the five year period and over varying climatic zones. When tested in the field, bacterized seeds could be stored dry for at least 2 years without losing the disease suppressing effect of the bacterial treatment.

3 Course of action – eradication methods

Additional information is provided by the IPPC (1998) in Guidelines for Pest Eradication Programmes. This standard describes the components of a pest eradication programme which can lead to the establishment or re-establishment of pest absence in an area. A pest eradication programme may be developed as an emergency measure to prevent establishment and/or spread of a pest following its recent entry (re-establish a pest free area) or a measure to eliminate an established pest (establish a pest free area). The eradication process involves three main activities: surveillance, containment and treatment and/or control measures.

As pathotypes of NFNB already occur in Australia, it may be sometime before an introduced pathotype was confirmed. Given that the feasibility of an eradication program will depend on early detection, delays in detecting new pathotypes may reduce the likelihood of developing a technically feasible, cost beneficial eradication Response Plan.

3.1 Destruction strategy

3.1.1 Destruction protocols

Infected crops should be destroyed by burning and ploughing. This will prevent aerial dispersal of the pathogen via infected crop residues. Infected trash may survive in the soil for several years and the paddock should not be re-cropped to barley or other susceptible hosts for at least three years.

The paddock may be cropped with pulses or oilseed crops for several years following the incursion and selective herbicides used to ensure the area remains free of barley and other potential host plants.

All vehicles and farm machinery that enter the infected field should be thoroughly washed, preferably using a detergent, farm degreaser or a 1% (available chlorine) bleach solution.

Any infected plant material or soil removed from the site should be incinerated, autoclaved or buried deeply (in a non-cropping area).

Unless the pathogen is detected very early it is unlikely that it can be eradicated. Fungal spores are likely to be carried into nearby crops. It can also survive on plant debris for several years. The pathogen is also likely to be transported over long distances via the movement of infested seed and contaminated vehicles and machinery.

3.1.2 Decontamination protocols

Machinery, equipment and vehicles in contact with infested plant material or soil or present within the Quarantine Area should be washed (or alternatively steam cleaned) to remove soil and plant material using high pressure water or scrubbing with products such as a farm degreaser or a 1% (available chlorine) bleach solution in a designated wash down. General guidelines for wash down areas are as follows:

- Located away from crops or sensitive vegetation
- Readily accessible with clear signage
- Access to fresh water and power
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting)
- Gently sloped to drain effluent away
- Effluent must not enter water courses or water bodies
- Allow adequate space to move larger vehicles
- Away from hazards such as power lines
- Waste water, soil or plant residues should be contained (see Appendix 18 of Plant Health Australia (2008))
- Disposable overalls and rubber boots should be worn when handling infested soil or plant material in the field. Boots, clothes and shoes in contact with infested soil or plant material should be disinfested at the site or double-bagged to remove for cleaning
- Skin and hair in contact with infested plant material or soil should be washed

3.1.3 Priorities

- Confirm the presence of the pathogen and determine whether it is likely to be exotic
- Prevent movement of vehicles and equipment through affected areas
- Priority of eradication/decontamination of infected host material
- Determine the extent of infection through survey and seed trace back
- Stop the movement of any seed that may be infected with *P. teres* f. sp. *teres*

3.1.4 Plants, by-products and waste processing

- Infected plant material should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial (in a non-cropping area)
- Crops or stubble should be destroyed by burning and deep ploughing
- Infected paddocks should remain free of host plants for at least three years

3.1.5 Disposal issues

- Once introduced and established, *P. teres* f. sp. *teres* can survive in infected crop residues and seed for long periods and thus be difficult to eradicate
- Particular care must be taken to minimize the transfer of soil or plant material from the area as NFNB can survive in soil on infected plant residues
- Raking and burning infected crops is not an option as this procedure is likely to spread the pathogen greater distances during the raking phase
- No particular issues with resistance of the pathogen to chemicals or physical treatments are known to exist

3.2 Quarantine and movement controls

3.2.1 Quarantine priorities

- Plant material and soil at the site of infection to be subject to movement restrictions
- Machinery, equipment, vehicles and disposable equipment in contact with infected plant material or soil to be subject to movement restrictions
- Harvesting of *P. teres* f. sp. *teres* infected crops should be prevented as the dust created during harvesting can spread the disease to neighbouring areas
- Wind-borne inoculum can escape from *P. teres* f. sp. *teres* infested crops, therefore the establishment of a quarantine area may be impractical

3.2.2 Movement control for people, plant material and machinery

Once symptoms of NFNB are observed the pathogen is usually well established in the crop and eradication difficult. Therefore, any zoning, quarantine or movement controls will usually pertain to containment and management.

If Restricted or Quarantine Areas are practical, movement of equipment or machinery should be restricted and movement into the area only occurs by permit. The industry affected will need to be informed of the location and extent of the disease occurrence.

Movement of people, vehicle and machinery, from and to affected farms, must be controlled to ensure that infected soil or plant debris is not moved off-farm on clothing, footwear, vehicles or machinery. This can be achieved through:

- Signage to indicate quarantine area and/or restricted movement in these zones

- Fenced, barricaded or locked entry to quarantine areas
- Movement of equipment, machinery, plant material or soil by permit only
- Clothing and footwear worn at the infected site should either be double-bagged prior to removal for decontamination or should not leave the farm until thoroughly disinfected, washed and cleaned
- All machinery and equipment should be thoroughly cleaned down with a pressure cleaner prior to leaving the affected farm. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine
- Seed from the affected site should not be used for planting new crops, feeding stock or for human consumption. Hay, stubble or trash should not be removed from the site.

3.3 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, biology of the pest, climatic conditions and the proximity of the infested property to other infested properties.

3.3.1 Destruction Zone

The entire crop should be destroyed after the level of infection has been established. The delimiting survey will determine whether or not neighbouring host crops are infected and need to be destroyed.

The Destruction Zone will usually be the entire crop but may be the entire farm or contiguous areas of management if spread is likely to have occurred prior to detection.

If the movement of air-borne inoculum to adjacent crops appears likely, they will also need to be destroyed.

Particular care needs to be taken to ensure that soils and plant material are not moved into surrounding areas not showing symptoms of disease. Where possible, destruction should take place in dry conditions to limit mud being spread within the field on boots and protective clothing.

3.3.2 Quarantine Zone

The Quarantine Zone is defined as the area where voluntary or compulsory restraints are in place for the affected property or properties. These restraints may include restrictions or movement control for removal of plants, people, soil or contaminated equipment from an infested property.

3.3.3 Buffer Zone

A Buffer Zone may or may not be required depending on the incident. It is defined as the area in which the pest does not occur but where movement controls or restrictions for removal of plants, people, soil or equipment from this area are still deemed necessary. The Buffer Zone may enclose an infested area (and is therefore part of the Control Area) or may be adjacent to an infested area.

3.3.4 Restricted Area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected premises. The Restricted Area is established following initial surveys that confirm the presence of the pathogen. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

3.3.5 Control Area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infested premises and all suspected infested premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

3.4 Decontamination and farm clean up

Decontamination practices are aimed at eliminating the pest thus preventing its spread to other areas.

3.4.1 Decontamination procedures

General guidelines for decontamination and clean up:

- Refer to PLANTPLAN, Appendix 18 (Plant Health Australia, 2008) for further information
- Keep traffic out of affected area and minimize it in adjacent areas
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms
- Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as detergent, farm degreaser or a 1% (available chlorine) bleach solution in a designated wash down area as described in 3.1.2
- Only recommended materials are to be used when conducting decontamination procedures, and should be applied according to the product label
- Plant material should be destroyed by high temperature incineration, autoclaving or deep burial (in a non-cropping area)

3.4.2 Decontamination if pest is identified in a small or large areas

Destruction of plant material should be carried out by burning or deep burial. The infected area would need to be monitored for 3 years for self sown plants which should be tested for NFNB and then destroyed.

3.4.3 General safety precautions

For any chemicals used in the decontamination, follow all safety procedures listed within each MSDS.

3.5 Surveillance and tracing

3.5.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Initial surveillance priorities include the following:

- Surveying all properties in the quarantine area with known hosts
- Surveying all properties identified in trace-forward or trace-back analysis as being at risk
- Surveying all host growing properties that are reliant on trade with interstate or international markets which may be sensitive to NFNB presence
- Surveying commercial grain traders that may have held infected seed

3.5.2 Survey regions

Establish survey regions around the surveillance priorities identified in Section 3.5.1. These regions will be generated based on the zoning requirements (see Section 3.3), and prioritised based on their potential likelihood to currently have or receive an incursion of this pest. Surveillance activities within these regions will either allow for the area to be declared pest free and maintain market access requirements or establish the impact and spread of the incursion to allow for effective control and containment measures to be carried out.

Steps outlined in Table 1 form a basis for a survey plan. Although categorised in stages, some stages may be undertaken concurrently based on available skill sets, resources and priorities.

Table 1. *Phases to be covered in a survey plan*

Phase 1	• Identify properties that fall within the buffer zone around the infected premise • Complete preliminary surveillance to determine ownership, property details, production dynamics and tracings information (this may be an ongoing action)
Phase 2	• Preliminary survey of host crops in properties in buffer zone establishing points of pest detection
Phase 3	• Surveillance of an intensive nature, to support control and containment activities around points of pest detection

Phase 4	• Surveillance of contact premises. A contact premise is a property containing susceptible host plants, which are known to have been in direct or indirect contact with an infected premises or infected plants. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the pest. Pathways to be considered are: o Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment o The producer and retailer of infected material if this is suspected to be the source of the outbreak o Labour and other personnel that have moved from infected, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers) o Movement of plant material and soil from Controlled and Restricted areas o Storm and rain events and the direction of prevailing winds that result in air-borne dispersal of the pest during these weather events
Phase 5	• Surveillance of private and public land where plants known to be hosts of *P. teres* f. sp. *teres* are being grown
Phase 6	• Agreed area freedom maintenance, pest control and containment

3.5.3 Post-eradication surveillance

The period of pest freedom sufficient to indicate that eradication of the pest has been achieved will be determined by a number of factors, including cropping conditions, the previous level of infection and the control measures applied.

Specific methods to confirm eradication of *P. teres* f. sp. *teres* may include:

- Monitoring of sentinel plants
    - Establishment of sentinel plants at the site of infection. Maintain good sanitation and hygiene practices throughout the year
    - Sentinel plants should remain in place and inspected on a fortnightly basis for the first 6 weeks and then on a monthly basis
    - Additional sentinel plants should be grown in pots using soils removed from the affected site. Plants are to be grown under quarantine containment glasshouse conditions and monitored for symptoms of infection
- Alternate non-host crops should be grown on the site and any self-sown cereals or grasses sprayed out with a selective herbicide
- Surveys comprising plant sampling for and testing for NFNB to be undertaken for a minimum of 3 years after eradication has been achieved

4 References

Anonymous (2008) Barley Variety Guide. *Victorian Department of Primary Industries, Agricultural Notes* AG 1099.

Bates JA, Taylor EJA (2001) Scorpion ARMS primers for SNP real-time PCR detection and quantification of *Pyrenophora teres*. *Molecular Plant Pathology* **2**, 275-280.

Brown MP, Steffenson BJ, Webster RK (1993) Host range of *Pyrenophora teres* f. *teres* isolates from California. *Plant Disease* **77**, 942-947.

Buchannon KW, McDonald WC (1965) Sources of resistance in Barley to *Pyrenophora teres*. *Canadian Journal of Plant Science* **45**, 189-193.

Cadorin ALR, Reis EM (2003) Eradication of *Drechslera teres* from barley seeds through fungicidal treatments. *Summa Phytopathologica* **29**, 199-201.

Cakir M, Gupta S, Platz GJ, Ablett GA, Loughman R, Emebiri LC, Poulsen D, Li CD, Lance RCM, Galwey NW, Jones MGK, Appels R (2003) Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.). (Better barley faster: the role of marker assisted selection). *Australian Journal of Agricultural Research* **54**, 1369-1377.

Campbell GF, Crous PW (2002) Fungicide sensitivity of South African net- and spot-type isolates of *Pyrenophora teres* to ergosterol biosynthesis inhibitors. *Australasian Plant Pathology* **31**, 151-155.

Douiyssi A, Rasmusson DC, Roelfs AP (1998) Responses of barley cultivars and lines to isolates of *Pyrenophora teres*. *Plant Disease* **82**, 316-321.

Ellis MB, Waller JM (1973) *Pyrenophora teres* (Conidial state: *Drechslera teres*). (*C.M.I. Descriptions of Fungi and Bacteria* No. 390: Kew, Surrey, UK).

Hampton JG, Matthews D (1980) The evaluation of seed-borne *Drechslera teres* in barley - a note on methodology. *Seed Science and Technology* **8**, 371-376.

Harrabi M, Kamel A (1990) Virulence spectrum to barley in some isolates of *Pyrenophora teres* from the Mediterranean region. *Plant Disease* **74**, 230-232.

Helm JH, Nyachiro JM, Cortez M, Oro M, Juskiw PE, Salmon DF (2004) Registration of 'Tyto' barley. *Crop Science* **44**, 343-344.

Hims MJ (1987) The effects of disease control on quality of winter barley. *Aspects of Applied Biology* **15**, 431-438.

Hollaway GJ (2009) Cereal Disease Guide. *Victorian Department of Primary Industries, Agricultural Notes* AG 1160.

Hollaway GJ, McLean MS (2008) Net blotches of barley. *Victorian Department of Primary Industries, Agricultural Notes* AG 1165.

IPPC (1995) ISPM No. 4: Requirements for the establishment of pest free areas. Secretariat of the International Plant Protection Convention, Rome.

IPPC (1998) ISPM No. 9: Guidelines for pest eradication programs. Secretariat of the International Plant Protection Convention, Rome.

Joergensen J (1977) Incidence of infections of barley seed by *Pyrenophora graminea* and *P. teres* as revealed by the freezing blotter method and disease counts in the field. *Seed Science and Technology* **5**, 105-110.

Johnsson L, Hokeberg 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**, 701-711.

Jonsson R, Bryngelsson T, Gustafsson M (1997) Virulence studies of Swedish net blotch isolates (*Drechslera teres*) and identification of resistant barley lines. *Euphytica* **94**, 209-218.

Jonsson R, Sall T, Kraft T, Gustafsson M (1999) Inheritance of resistance to *Pyrenophora teres* f. *teres* in spring barley. *Plant Breeding* **118**, 313-317.

Jordan VWL (1981) Aetiology of barley net blotch caused by *Pyrenophora teres* and some effects on yield. *Plant Pathology* **30**, 77-87.

Jordan VWL, Allen EC (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**, 547-559.

Jordan VWL, Best GR (1984) Action of fungicides against barley net blotch, *Pyrenophora teres*. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology,* Supplement) **104***,* 44-45.

Keiper FJ, Grcic M, Capio E, Wallwork H (2008) Diagnostic microsatellite markers for the barley net blotch pathogens, *Pyrenophora teres* f. *maculata* and *Pyrenophora teres* f. *teres*. *Australasian Plant Pathology* **37**, 428-430.

Khan TN, Boyd WJR (1969) Inheritance of resistance to net blotch in barley. II. Genes conditioning resistance against race W.A.2. Canadian *Journal of Genetics and Cytology* **11**, 592-579.

Leisova L, Kucera L, Minarikova V, Ovesna J (2005) AFLP-based PCR markers that differentiate spot and net forms of *Pyrenophora teres*. *Plant Pathology* **54**, 66-73.

Leisova L, Minarikova V, Kucera L, Ovesna J (2006) Quantification of *Pyrenophora teres* in infected barley leaves using real-time PCR. *Journal of Microbiological Methods* **67**, 446-455.

Martin RA (1985) Disease progression and yield loss in barley associated with net blotch, as influenced by fungicide seed treatment. *Canadian Journal of Plant Pathology* **7**, 83-90.

Martin RA, Clough KS, Johnston HW (1984) Vertical and horizontal distribution of conidia of *Pyrenophora teres* in barley fields. *Canadian Journal of Plant Pathology* **6**, 280-282.

McLean MS, Howlett BJ, Hollaway GJ (2009) The epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. *Australasian Plant Pathology* (In Press).

Merriman P, McKirdy S (2005) Technical guidelines for the development of pest specific response plans, Plant Health Australia.

Piening L (1968) Development of barley net blotch from infested straw and seed. *Canadian Journal of Plant Science* **48**, 623-625.

Plant Health Australia (2008) PLANTPLAN Australian Emergency Plant Pest Response Plan. Version 1. (**www.planthealthaustralia.com.au/plantplan**).

Platz G, Bell KL, Rees RG, Galea VJ (2001) Proceedings of the 8th International Barley Genetics Symposium, Vol. 2, pp. 160-162.

Robinson J, Jalli M (1996) Diversity among Finnish net blotch isolates and resistance in barley. *Euphytica* **92**, 81-87.

Robinson J, Mattila I (2000) Diversity and difference within and between *Pyrenophora teres* f. *teres* populations measured with Kosman's indices. *Archives of Phytopathology and Plant Protection* **33**, 11-23.

Sato K, Takeda K (1990) Studies on the conidia formation of *Pyrenophora teres* Drechs. 1. Effects of medium, temperature and light quality. *Nogaku Kenkyu* **62**, 151-163.

Serenius M (2006) Population structure of *Pyrenophora teres*, the causal agent of net blotch of barley (Doctoral Dissertation). *Agrifood Research Reports* **78**, 1-60.

Serenius M, Manninen O (2006) Prochloraz tolerance of *Pyrenophora teres* population in Finland. *Agriculture and Food Science* **15**, 35-42.

Serenius M, Manninen O, Wallwork H, Williams K (2007) Genetic differentiation in *Pyrenophora teres* populations measured with AFLP markers. *Mycological Research* **111**, 213-223.

Shaw MW (1986) Effects of temperature and leaf wetness on *Pyrenophora teres* growing on barley cv. Sonja. *Plant Pathology* **35**, 294-309.

Sheridan JE (1977) *Drechslera* spp. and other seed-borne pathogenic fungi in New Zealand cereals. *New Zealand Journal of Agricultural Research* **20**, 91-93.

Sheridan JE, Grbavac N, Sheridan MH (1985) Triadimenol insensitivity in *Pyrenophora teres*. *Transactions of the British Mycological Society* **85**, 338-341.

Shipton WA, Khan TN, Boyd WJR (1973) Net blotch of barley. *Review of Plant Pathology* **52**, 269-290.

Steffenson B (1997) Net blotch. In '*Compendium of Barley Diseases*'. (Ed. D Mathre) pp. 28-31. (The American Phytopathological Society: St. Paul, Minnesota).

Steffenson BJ, Webster RK (1992) Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. *Phytopathology* **82**, 170-177.

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**, 141-148.

Tekauz A, Buchannon KW (1977) Distribution of and sources of resistance to biotypes of *Pyrenophora teres* in Western Canada. *Canadian Journal of Plant Science* **57**, 389-395.

Thomas JE, Reeves JC, Taylor EJA, Kenyon DM (1998) The development of new diagnostic techniques and their role in improving treatment strategies for seed-borne diseases. In '*Proceedings Brighton Crop Protection Conference: Pests & Diseases - 1998*'. Brighton, UK pp. 787-792.

Turkington TK, Xi K, Clayton GW, Burnett PA, Klein-Gebbinck HW, Lupwayi NZ, Harker KN, O'Donovan JT (2006) Impact of crop management on leaf diseases in Alberta barley fields, 1995-1997. *Canadian Journal of Plant Pathology* **28**, 441-449.

van den Berg CGJ (1988) Epidemiology of *Pyrenophora teres* and its effect on grain yield of *Hordeum vulgare*. Ph.D., University of Saskatchewan.

van den Berg CGJ, Rossnagel BG (1990) 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.

Weiland JJ, Steffenson BJ, Cartwright RD, Webster RK (1999) Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. *Phytopathology* **89**, 176-181.

Williams KJ, Smyl C, Lichon A, Wong KY, Wallwork H (2001) Development and use of an assay based on the polymerase chain reaction that differentiates the pathogens causing spot form and net form of net blotch of barley. *Australasian Plant Pathology* **30**, 37-44.

Wu HL, Steffenson BJ, Li Y, Oleson AE, Zhong S (2003) Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. *Canadian Journal of Plant Pathology* **25**, 82-90.

4.1.1 4.1 Websites

AQIS import conditions database (**www.aqis.gov.au/icon32/asp/ex_querycontent.asp)**

Barley diseases, Queensland DEEPI (**www.dpi.qld.gov.au/cps/rde/dpi/hs.xsl/26_3528_ENA_HTML.htm**)

CAB Compendium (**www.cabicompendium.org/cpc/home.asp**.)

Net blotches of barley, Victorian DPI (**www.dpi.vic.gov.au/DPI/nreninf.nsf/v/4764423FCB04DD67CA25745D0000DD45/$file/Net_Blotches_of_Barley.pdf**)

Victorian DPI (**www.dpi.vic.gov.au**)

Winter crop variety sowing guide 2009, NSW DPI (**www.dpi.nsw.gov.au/agriculture/field/field-crops/winter-cereals/guides/winter-crop-variety-sowing-guide**)

5 Appendices

Appendix 1. Standard diagnostic protocols

For a range of specifically designed procedures for the emergency response to a pest incursion refer to Plant Health Australia's PLANTPLAN (**www.planthealthaustralia.com.au/plantplan**).

Appendix 2. Experts, resources and facilities

The following tables provide lists of experts (Table 2) and diagnostic facilities (Table 3) for use in professional diagnosis and advisory services in the case of an incursion.

Table 2. *Experts who can be contacted for professional diagnostic and advisory services*

Expert	State	Details
Dr Hugh Wallwork	SA	SARDI Plant Research Centre Waite Main Building, Waite Research Precinct Hartley Grove Urrbrae SA 5064 Ph: (08) 8303 9400; Fax: (08) 8303 9403
Greg Platz	QLD	QLD Department of Primary Industries Hermitage Research Station 604 Yangan Rd Warwick Ph (07) 4660 3633 ; Fax (07) 4660 3600
Sanjiv Gupta	WA	Department of Agriculture and Food, WA 3 Baron-Hay Court South Perth Ph (08) 9368 3188; Fax (08) 9474 2840
Mark McLean	VIC	DPI Victoria Horsham Centre Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187

Table 3. *Diagnostic service facilities in Australia*

Facility	State	Details
DPI Victoria Knoxfield Centre	Vic	621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9222; Fax: (03) 9800 3521
DPI Victoria Horsham Centre	Vic	Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187

Facility	State	Details
DPI New South Wales, Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: (02) 4640 6327; Fax: (02) 4640 6428
DPI New South Wales, Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
DPI New South Wales, Wagga Wagga Agricultural Institute	NSW	PMB Wagga Wagga NSW 2650 Ph: (02) 6938 1999; Fax: (02) 6938 1809
SARDI Plant Research Centre - Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae SA 5064 Ph: (08) 8303 9400; Fax: (08) 8303 9403
Grow Help Australia	QLD	Entomology Building 80 Meiers Road Indooroopilly QLD 4068 Ph: (07) 3896 9668; Fax: (07) 3896 9446
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: (08) 9368 3721; Fax: (08) 9474 2658

Appendix 3. Communications strategy

A general Communications Strategy is provided in Appendix 6 of PLANTPLAN (Plant Health Australia, 2008).

Any report of disease outbreaks in resistant cultivars should be investigated immediately to establish whether there has been a change in the virulence of the pathogen or an incursion by a more virulent strain of the fungus.

The grains industry also needs to be informed that a suspected incursion of a more virulent strain of *P. teres* f. sp. *teres* has occurred.

Appendix 4. Market access impacts

Within the AQIS PHYTO database, no countries appear to have a specific statement regarding area freedom from *P. teres* f. sp. *teres* (April 2009). Should *P. teres* f. sp. *teres* be detected or become established in Australia, some countries may require specific declaration. Latest information can be found within PHYTO, using an Advanced search “Search all text” for *Pyrenophora teres* f. sp. *teres.*