# INDUSTRY BIOSECURITY PLAN
# FOR THE GRAINS INDUSTRY

## Threat Specific Contingency Plan

# Leaf blight of wheat

## *Alternaria triticina*

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**and Plant Health Australia**

**August 2009**

Plant Health AUSTRALIA

GRDC Grains Research & Development Corporation

CRC PLANT biosecurity

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## Further information

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


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# 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 leaf blight of wheat (*Alternaria triticina*). It provides guidelines for steps to be undertaken and considered when developing a Response Plan to this pest. Any Response Plan developed using information in whole or in part from this Contingency Plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

# 2 Pest information/status

## 2.1 Pest details

*Alternaria triticina* (Prasada & Prabhu, 1962)

Common names: Leaf blight of wheat, Alternaria leaf blight

### 2.1.1 General information

Taxonomic position – Phylum: Ascomycota; Class: Ascomycetes; Order: Pleosporales; Family: Pleosporaceae

Leaf blight of wheat is caused by the fungus *Alternaria triticina*. This fungal disease infects wheat species and a small number of related cereals. Infection occurs through seed borne transmission, planting into infected soil, or from infested crop residues, where rain splash or leaves coming in direct contact with the soil leads to infection. *A. triticina* is unable to infect young wheat seedlings under about four weeks of age, with symptoms not seen until plants are about seven weeks of age. Susceptibility increases with plant age.

Infected plants present symptoms starting as small lesions on the leaves, which grow in size over time. Under heavy pathogen load the infection and symptoms can spread to the leaf sheaths and to seed heads where it can have a detrimental effect on seed quantity and quality.

Survival of *A. triticina* in the soil is limited, with hot and wet summer conditions killing off fungi present in the soil (Kumar & Arya, 1973, Kumar & Rao, 1979b). Survival of the fungus in plant debris can occur but debris left on the soil surface will survive only half as long as that buried in the soil (two versus four months; Kumar & Rao, 1979b). Due to these considerations, seed borne transmission of *A. triticina* is the most likely mechanism, as it may not survive in the soil or on plant debris until the planting of the next crop. However, if conditions are dry between successive wheat crops, the fungus may survive on infested residues.

Disease severity can be affected by the levels of nitrogen and phosphorus present in the system. There are reports of both negative (Rashid *et al.,* 1985) and positive (Ram & Joshi, 1981, Logrieco *et al.,* 1990) relationships between disease severity and nitrogen and phosphorus levels.

### 2.1.2 Disease cycle

Inoculum, in the form of spores, either survives in the soil during absence of hosts or is carried on seeds. Seed borne inoculum multiplies and becomes established in the soil following planting, and it is from here that the pathogen infects the plant once it reaches a susceptible stage. Leaves become infected either through contact with the ground or rain splash transfers inoculum to the lower leaves. From here the pathogen slowly transfers to other leaves.

Temperatures of 20–25°C are optimal for infection and disease development, with 10 h of continuous leaf wetness required for infection (Prabhu & Prakash, 1973), although longer high humidity over 90% for 48 h is optimal. Spores germinate on the surface of the leaves (both upper and lower) producing up to 4 germ tubes. These produce appressoria on the leaf surface and directly infect through the epidermis. Occasionally the infection occurs through the stomata.

Following penetration, hyphae grow through the leaf tissue b oth intra- and extra-cellularly. Hyphae infect cells directly beneath the epidermis and the parenchyma cells, flattening them, but will not infect vascular tissue. The flattening of the cells results in a substantial reduction in leaf thickness. *A. triticina* produces non-host specific toxins that contribute to cell death and collapse, and the development of the lesions (Kumar & Rao, 1979a).

Sporulation occurs abundantly in older lesions, with conidia disseminated by wind that provide secondary inoculum for disease spread. In Uttar Pradesh, India, conidia are present in air during the rainy season but were not detected in the dry summer or winter (Singh *et al*. 2005). In Maharashtra, conidia were mostly dispersed during February and early March (wheat at grain filling stage) with minimum temperature, wind speed and relative humidity positively correlated with number of spores (Rathod *et al*., 2006). In West Bengal, air-borne conidia were most abundant with increasing temperatures above 30°C (minimum 20°C) and wind velocity above 5 km/h (Maity & Srikanta Das, 2003).

Late season infection of the heads leads to seed infection, with seed-borne inoculum being the most important primary inoculum to initiate infections in the next crop (Anahosur, 1978). Conidia from infected residues can be important with short rotations, but the fungus does not survive long in residues or in the soil under hot, wet conditions (Kumar & Rao, 1979b).

## 2.2 Affected hosts

### 2.2.1 Host range

The host range is confined to *Triticum* spp. and triticale, with a possibility that barley is infected under some circumstances (Table 1).

***Table 1.*** *Host range of leaf blight of wheat*

| | |
|---|---|
| **Major hosts** | *Triticum aestivum* (wheat), *T. turgidum* ssp. *durum* (durum wheat), *X Triticosecale* (triticale) |
| **Minor hosts** | *Avena sativa* (oats)[1], *T. dicoccum*, *T. sphaerococcum* (dwarf wheat), *Hordeum vulgare* (barley)[1], *Secale cereal* (rye)[1], *Musa* (banana)[2] |

[1] Varying reports of these being hosts so their status is uncertain

[2] Only one report. Given the current opinion on species in the genus *Alternaria*, the record on *Musa* (banana) is unlikely (see discussion in Section 2.4.1).

### 2.2.2 Geographic distribution

*A. triticina* has been identified in the following countries:

- Asia: Bangladesh, India, Israel, Lebanon, Pakistan, Turkey, Yemen
- Europe: France, Greece, Italy, Macedonia, Portugal
- Africa: Egypt, Nigeria
- North America: Mexico
- South America: Argentina (Perellò & Sisterna, 2006)
- Oceania: Australia (Jones, 1991)[3]

### 2.2.3 Symptoms

Young plants are resistant to the pathogen. Seedlings begin to lose this resistance at about four weeks of age and symptoms usually do not develop until the plants have reached 7–8 weeks of age. The lowermost leaves are always the first to show symptoms, and the disease will gradually spread to the higher leaves.

Early symptoms are small, oval, chlorotic lesions that are irregularly scattered on the leaves. These enlarge, become irregular in shape, and develop into dark brown or grey sunken lesions. Lesions may have a bright-yellow marginal zone, and can grow up to 1 cm or more in diameter. Lesions may coalesce and cover large regions of the leaf (Figure 1), resulting in the death of the whole leaf. Under moist conditions, lesions may be covered by black powdery conidia. Under severe conditions the symptoms can also be seen on the leaf sheath, awns and glumes.

At the dough stage of wheat development, heavily infected fields appear dull and bronzed with a burnt appearance when seen from a distance (Prabhu & Prasada, 1966).

Symptoms by affected plant part:

- Growing points: lesions
- Inflorescence: lesions
- Leaves: lesions, abnormal colours and fungal growth
- Seeds: discolouration and shrivelled

Similarity to other wheat diseases:

- Bipolaris leaf spot, caused by *Cochliobolus sativus* (= *Bipolaris sorokiniana*). Alternaria leaf blight often occurs with this leaf spot in India.
- Yellow spot, caused by *Pyrenophora tritici-repentis*. Lesions of yellow spot on resistant wheats appear similar to young lesions of Alternaria leaf blight.

[3] The Australian record is based on isolation of *A. triticina* from rotting banana crowns (part of fruit attached to the bunch) from East Palmerston, Northern Queensland. Current taxonomic opinion on the genus *Alternaria* casts doubt on the banana isolate being *Alternaria triticina* (see Section 2.4.1). However, if it was the wheat attacking form of *A. triticina*, and since bananas from North Queensland are distributed throughout Australia for sale, there would have been ample opportunity for this fungus to have spread.

***Figure 1.*** *Symptoms of A. triticina on wheat leaves and seeds. Figure taken from Perellò & Sisterna, 2006.*

## 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, Leaf blight was given an overall risk rating as High and as a consequence, this contingency plan was commissioned. During preparation of the contingency plan, literature searches undertaken by the author have shown the ratings for economic potential and spread potential to be less thus reducing the overall risk.

### 2.3.1 Entry potential

**Rating: High**

*Alternaria triticina* is readily seed-borne on wheat and durum. Thus, entry potential is high if wheat seed were introduced from areas where the pathogen occurs. Such seed could enter as:

- Direct imports of wheat or durum seed for breeding purposes or consumption
- Contaminants in bulk commodities, agricultural machinery and some bulk feed grains
- Inadvertent entry (intentional or unintentional with travellers' goods)

There is a high frequency of travel between areas in India and Italy where the pathogen exists and Australian farming areas.

### 2.3.2 Establishment potential

**Rating: Medium**

The rate of seed transmission is high so infected seed sown in an area suitable for development of Alternaria leaf blight would have a high potential for establishing the pathogen. However, the present wheat growing areas of Australia appear unsuitable for development of this leaf blight. In India, Alternaria leaf blight often occurs with Bipolaris leaf spot caused by *Cochliobolus sativus* (*Bipolaris sorokiniana*). This latter pathogen occurs throughout the Australian wheat belt where it causes a root rot, but the leaf spot is seldom observed (Murray & Brennan, 2009). Bipolaris leaf blight does occur in very high humidity conditions in coastal NSW (Murray & Smith, 1970). In the absence of more detailed epidemiological analysis, the absence of Bipolaris leaf spot is evidence that conditions are also not suitable for Alternaria leaf blight. However, if wheat were to be produced in high humidity, warm coastal areas of Queensland and northern NSW, Alternaria leaf blight may have a high establishment potential.

### 2.3.3 Spread potential

**Rating: High**

There is a high rate of seed transmission by *A. triticina* so the pathogen would be readily distributed with infected seed.

Spores of *Alternaria* spp. are major components of the air-borne microflora. However, there is evidence that air-borne spread of *A. alternata* is mostly short distances within cotton crops, so that most spread would be confined within the affected crop, with limited spread to neighbouring crops (Bashan et al., 1991). If this applies to *A. triticina*, such spread would introduce the pathogen to neighbouring crops initially at a very low level.

### 2.3.4 Economic impact

**Rating: Low - Medium**

Production: Alternaria leaf blight causes major yield and quality loss in affected crops but conditions in the southern and western Australian wheat belts appear unsuitable for development of this disease. Bipolaris leaf spot, which usually occurs with Alternaria leaf blight in India, causes no loss to Australian wheat (Murray & Brennan, 2009).

Trade: the presence of *A. triticina* in Australia wheat could affect marketing to some overseas countries (see Appendix 4) with consequent effects on the price received. The countries presently imposing regulations are New Zealand, Brazil and South Africa, which are not major buyers of Australian wheat. They would require area freedom statements.

If wheat production occurred in high humidity, warm areas of Australia, the potential yield loss would be high. In such areas of India, yield losses of more than 50% can occur, with losses around 20% common (Singh *et al*., 2002).

### 2.3.5 Environmental impact

**Rating: Negligible**

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

### 2.3.6 Overall risk

**Rating: Low - Medium**

Although there is a high risk of introduction, the risks of establishment and economic loss are low.

## 2.4 Diagnostic information

### *2.4.1* Characteristics of *Alternaria triticina*

*Alternaria triticina* occurs on *Triticum* spp., triticale and barley while inoculation has failed to show its virulence on eight Poaceae species or on other plants. Identification of *Alternaria* species by morphological characters requires a combination of rigorous methods including the three-dimensional sporulation pattern of the small-spored isolates.

*A. triticina* belongs to sporulation group 6, the *A. infectoria* species group. Toxin production, PCR and nucleotide sequencing molecular analysis, and the pathogenicity to wheat are additional characters useful for distinguishing *A. triticina* from other *Alternaria* species (Mercado Vergnes *et al.,* 2006).

These recent findings cast doubt on some earlier reports of *A. triticina* (Mercado Vergnes *et al*., 2006). For Australia, the *Alternaria* isolate from banana crowns was identified by the International Mycological Institute, Kew, U.K. as *A. triticina*. It caused moderately severe damage when inoculated onto banana crowns (Jones, 1991) but there were no reports of inoculations onto wheat. Thus, the identity of the fungus isolated from North Queensland bananas remains in question. Until definitive work is done on the banana *Alternaria*, bananas should not be regarded as a host of *A. triticina* and there is no conclusive record of this species from Australia.

### 2.4.2 Diagnostic protocol

Identification of *A. triticina* is achieved through morphological features of the pathogen (Anahosur, 1978), pathogenicity studies and sequencing (Mercado Vergnes *et al*., 2006) and should be completed by an expert.

The pathogen can be isolated by surface sterilisation of infected plant parts and plating out on agar. Alternatively, infected tissue can be incubated at high humidity to encourage sporulation and conidia can be picked with a needle and placed on agar. Isolates from tissue or conidia are grown on nutrient agar, such as standard nutrient agar, potato dextrose agar or potato carrot agar. The mycelium is initially hyaline and becomes a deep olive buff. Growth is optimal between 20 and 24°C (Weise 1987). Potato carrot agar is the standard to characterise conidial morphology and sporulation pattern group (Simmons, 1992; Mercado Vergnes *et al*., 2006), with standard nutrient agar the best growth medium for sporulation.

*A. triticina* is distinguished by its wheat-specific virulence and somewhat by its morphological characters.

Description of conidia (Figure 2):

- Colour: light brown to olive and become darker with age
- Shape: Irregularly oval, ellipsoid conical, gradually tapering into a beak
- Dimensions: 15-92 x 8-35 µm
- Septa: 1-10 transverse and 0-5 longitudinal

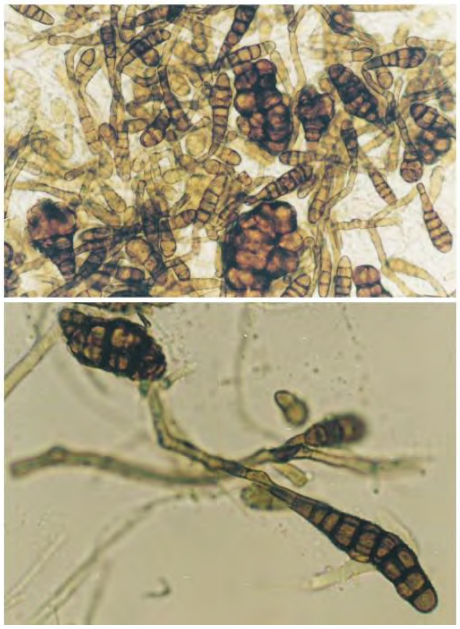

***Figure 2.*** *Morphology of the conidia of A. triticina isolated from wheat (magnification 400x). Figure taken from Perellò & Sisterna, 2006.*

## 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

Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas to poor growth. The normal procedure is to collect symptomatic plants and to test them to confirm the presence of *Alternaria* sp. 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 and trace-forward 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. 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 disease distribution. Depending on the stage of infection the symptoms may appear as (see Section 2.2.3 full details):

- Plants with leaf lesions
- Plants with premature defoliation
- Patches of plants dying within the crop

It is important to note the distribution of disease in the initial crop, as this will indicate whether the disease 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.

Any personnel collecting leaf 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 PLANTPLAN, Appendix 3 (Plant Health Australia 2008a).

#### *2.6.1.1 NUMBER OF SPECIMENS TO BE COLLECTED*

The initial outbreak will appear as small to larger lesions on plants in groups within the planting. These will be associated with spread from the initial seed-borne infection. If only a small area is affected, all plants with symptoms should be collected. If there are several foci of infection, collect up to 10 plants with a range of symptoms from up to 10 locations within the affected planting.

#### *2.6.1.2 HOW TO COLLECT*

Leaves are the main organ infected with *Alternaria* sp. However under heavy infection and humid conditions the fungus can infect leaf sheaths and seeds. Samples should be collected 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.

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 that 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 and emergency pathogens are undertaken according to the following parameters:

- The laboratory diagnostician has expertise in this form of diagnosis
- The test is undertaken as described in Section 2.4.2
- The results are confirmed by diagnosis in another recognised laboratory or by another diagnostician
- Where possible, diagnosis is confirmed by a second method

#### *2.6.1.3 HOW TO PRESERVE PLANT SAMPLES*

Infected plant samples should be stored in a cool (4°C) moist environment when to be used for subculturing fungi, or frozen at -80°C for DNA analysis. See above for details.

#### *2.6.1.4 HOW TO TRANSPORT PLANT SAMPLES*

Suspect samples should be marked “Plant Sample for Urgent Diagnosis” and sent to the nearest diagnostic laboratory (see Appendix 2 of this document for addresses and Appendices 3-5 of PLANTPLAN for collection and transport of samples (Plant Health Australia, 2008)).

Green plant samples should be wrapped in moist but not wet paper and placed in a suitable plastic bag. Grain samples need to be tightly packed into a plastic container (preferably) or in a plastic bag.

Double bag the samples and wipe the outside of the bag with alcohol and allow to dry before dispatching the sample to the laboratory.

Additional information including the detail of the sample date, location and site must be recorded on an accompanying sheet, together with all relevant paperwork. This information should be placed in a

plastic bag, on which is also written the summary details of the sample and the address, and included with the samples that are dispatched.

All samples should be dispatched using an overnight courier service or Express Post.

Important: Prior to dispatch, the Manager of the laboratory to which the sample is being consigned should be advised by telephone (not e-mail – a more direct advice than e-mail is required) of the expected arrival date. Special arrangements may need to be made for weekends. If the receiving laboratory is in another state, then a permit from AQIS is required for the movement of seed into that State. Check with the State or Local Pest and Disease Control Headquarters that approval has been granted.

See PLANTPLAN for further details of sampling and transport (Plant Health Australia, 2008a).

### 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 disease to spread from 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 crops have been sown from the same source seed
- The proximity of other susceptible crops to the initial infected crop, both in the current growing season and previous season. This will include the growers own crops and those 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

Some general comments about possible mechanisms of spread are:

- Movement of infected seed. The pathogen has the potential to be transmitted as infected seed. Small infected fragments can also be carried within infested seed lots. Initial infections will be in small patches around the seed carrying the fungus, and these will be random within the planting
- Mechanical transmission through movement on contaminated vehicles and machinery. The initial infections may be associated with the first point of entry into the field
- Small fragments of plant debris and spores released from infested plant debris can be blown into surrounding paddocks during harvesting and allow the pathogen to move considerable distances away from the infected crop. The initial infections will usually show a gradient with highest incidence along the side of the planting closest to the source of inoculum
- Fungal spores that adhere to clothing, machinery or animals can be carried large distances into other wheat crops. The initial infections may be associated with the first point of entry into the field

### 2.6.4 Pest Free Area (PFA) guidelines

Points to consider are:

- Design of a statistical delimiting field survey for symptoms on host plants (see section 2.6.1 for points to consider in the design)
- Plant sampling should be based on at least 100 plants taken at random from each crop
- Seed sampling should be based on a minimum of 400 seeds as infection levels in seeds can be low
- Surveys should also consider alternative hosts (see Section 2.2.1) and not be limited to the primary infected host
- Survey around irrigation systems or waterways that may have transported spores. Also, the high humidity around these will provide the most likely conditions for infection

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 pest free areas as a risk management option for phytosanitary certification of plants and plant products. Establishment and maintenance of a PFA can vary according to 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).

## 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
- Stop irrigating affected (irrigated crops) areas and use bunding to divert overland flood flows around them (both irrigated and dryland crops)
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms
- After surveys are completed, destruction of the infected crop is an effective control (see Section 3.1.1)
- On-going surveillance of infected paddocks to ensure leaf blight is eradicated
- Ensure that planting seed production does not take place on affected farms and do not use seed from these farms to plant next crop as leaf blight can be seed borne
- Zero-tillage can be used to take advantage of the lower survival of *Alternaria* on the soil surface in comparison to plants buried in the soil
- Do not grow susceptible plants in infected fields for at least two years following eradication of the disease

### 2.7.2 Control if small areas are affected

Collect all plants in the area into bags and destroy by burning or burial. Do not sow any *Triticum* spp. or triticale in the area for two years.

### 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.

Given the low overall risk posed by this pathogen, if confirmed across large areas, eradication would be unlikely to be cost beneficial.

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 seed or another source (e.g., contaminated clothing or machinery). If the disease is found to be confined to a single seed lot and only found in a specific crop species, it may be possible to eradicate the disease by destroying all crops of that type in the region.

If eradication was attempted, there would need to be ongoing monitoring of infected paddocks to ensure there was no opportunity for the pathogen to re-establish on self sown plants.

### 2.7.4 Specific controls for *A. triticina*

#### *2.7.4.1 CULTURAL CONTROL*

Pathogen levels on seeds can be reduced through hot water treatment (Prabhu & Prasada, 1970). Pre-soaking seeds in ambient temperature water for 4 hours, followed by a hot water treatment (52–54°C for 10 minutes) effectively controls fungus levels without adversely affecting seed viability.

Carboxin, registered in Australia for seed treatment of wheat, gives some control of seed-borne infection by *A. triticina* (Singh *et al*., 2007).

The fungus can survive on plant debris for several months and can serve as a source of infection. A zero-tilling approach leaving the plant debris on the surface of the soil will reduce the length of fungus survival. The hot, moist conditions presented on the soil surface in wet environments reduce the length of survival to below 2 months, while burial allows for the survival for over 4 months (Kumar & Rao, 1979b). Susceptible host plants should not be replanted into the field directly following eradication of the disease. Crop rotation with resistant species or leaving the field bare for two years should occur to break the disease cycle. Any self sown susceptible plants should be removed to stop them acting as disease reservoirs.

Crop rotation with rice eliminates *A. triticina* from the field (Ahmad & Imran, 2008).

#### *2.7.4.2 HOST PLANT RESISTANCE*

Wheat host-plant resistance to leaf blight is highly variable across wheat genotypes. In general, bread wheat varieties have a greater resistance to *A. triticina* than durum or dwarf wheat varieties (Prabhu & Prasada, 1966). However, the large number of resistant lines available provides a large amount of material for breeding resistance into any specific genotype.

The mechanism of host-plant resistance to *A. triticina* is not yet determined, but a number of correlations have been observed between physiological characteristics and resistance levels. Infection results in a reduction in the sugar, starch and phenolic content of the leaves, but phenolic levels of resistant lines increase in response to infection (Kumar & Rao, 1980). Resistant cultivars also show higher levels of phenolics and peroxidase activity before infection in comparison to susceptible lines (Tyagi *et al.,* 1998). In contrast, high levels of sugars are seen in susceptible cultivars (Kulshrestha & Rao, 1977). In addition, levels and composition of cuticular wax correlate with resistance (Kumar, 1974), possibly inhibiting entry of the pathogen following germination of the

spore on the leaf surface. This relationship is not purely based on cuticular thickness (Kulshrestha & Rao, 1978).

Plant resistance seems to directly affect the growth patterns of the pathogen. *A. triticina* does not penetrate leaves of resistant plants with limited spore tube development. On susceptible plants the germ tube growth was extensive with penetration of almost all cells occurring (Kumar *et al.,* 1974).

The genetics of resistance to *A. triticina* in wheat are highly variable, with a number of different genes and gene combinations providing resistance in different varieties. There is evidence for a single dominant gene providing resistance (Kulshrestha & Rao, 1976), while in other cases two independent dominant (Narula, 1982), two complimentary dominant (Kulshrestha & Rao, 1976) or two recessive (Sokhi *et al.,* 1973) genes provide resistance. Currently, no *A. triticina* resistance gene has been identified and sequenced.

#### *2.7.4.3 CHEMICAL CONTROL*

*A. triticina* can be controlled through the application of fungicides, with a 75% reduction in disease severity and an increase in plant yield observed following some treatments. However, the fungus is also known to develop resistance over time to certain fungicides (Sankhla *et al.,* 1970) and multiple applications with more than one type of fungicide are required for effective control.

Fungicides that have previously been shown to be effective against *A. triticina* are mancozeb, ziram, zineb, thiram, fytolan, propineb, chlorothalonil and nabam. Propiconazole and tebuconazole are also effective (Diaz Franco & Rodriguez Campos, 1998). The pathogen can also be removed from seeds through a seed based fungicide application.

#### *2.7.4.4 MECHANICAL CONTROL*

There are no mechanical controls for Alternaria leaf blight.

#### *2.7.4.5 BIOLOGICAL CONTROL*

Several isolates of fluorescent pseudomonads and *Bacillus* spp. are effective in suppressing infection of wheat plants when applied as soil treatments (Siddiqui, 2007).

#### *2.7.4.6 INTEGRATED CONTROL*

Combinations of fertilizers, seed treatment, foliar sprays of fungicide, and bio-control agents gave effective control of the leaf blight complex caused by *C. sativus* and *A. triticina* (Singh *et al*., 2008).

# 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.

## 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
- Herbicides can be used to destroy the infected crops. Knockdown herbicides will not prevent some additional development of *A. triticina* on killed tissue since the fungus probably has good saprophytic ability. Dessication herbicides, while not reducing the fungus, would prepare the crop residues for burning.
- Infested fields should not be resown to wheat for a minimum of two years. The first and second wheat crops sown after this time should be monitored for *A. triticina*.
- Disposable equipment, infected plant material or soil should be disposed of by autoclaving, high temperature incineration or deep burial
- Any equipment removed from the site for disposal should be double-bagged

### 3.1.2 Decontamination protocols

If containment, eradication and/or best practice hygiene measures are implemented, 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 a farm degreaser or a 1% bleach (available chlorine) 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 PLANTPLAN 2008 Appendix 18)
- Disposable overalls and rubber boots should be worn when handling infected soil or plant material in the field. Boots, clothes and shoes in contact with infected soil or plant material should be disinfected 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

Specific priorities for eradication:

- Confirm the presence of the pathogen
- 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 the pathogen

### 3.1.4 Plants, by-products and waste processing

- Seeds harvested from infected plants and any soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial (in a non-cropping area)
- As the pathogen can be mechanically transmitted, killed crops should be ploughed in or burnt
- Infested paddocks should remain free of susceptible host plants until soil has been shown to be free from the pathogen

### 3.1.5 Disposal issues

- Particular care must be taken to minimise the transfer of infected soil or plant material from the area
- Raking infected crops is not an option as this procedure is likely to spread the pathogen greater distances during the raking process
- No particular issues with resistance of disease 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 infestation to be subject to movement restrictions
- Machinery, equipment, vehicles and disposable equipment in contact with infested plant material or soil to be subject to movement restrictions
- Harvesting of infected crops should be prevented as the dust created during harvesting can spread the disease to neighbouring areas

### 3.2.2 Movement control for people, plant material and machinery

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
- Hay, stubble or trash must not be removed from the site
- 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 (see Section 3.1.2)
- Seed from the affected site should not be used for planting new crops, feeding stock or for human consumption

## 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 infected property to other infected properties.

### 3.3.1 Destruction Zone

The size of the destruction zone (i.e. zone in which the pest and all host material is destroyed) will depend on the ability of the pest to spread, distribution of the pest (as determined by delimiting surveys), time of season (and part of the pest life cycle being targeted) and factors which may contribute to the pest spreading.

The entire crop or pasture should be destroyed after the level of infestation has been established. The delimiting survey will determine whether or not neighbouring host crops are infested and need to be destroyed. The Destruction Zone may be defined as contiguous areas associated with the same management practices as the infested area (i.e. the entire trial, paddock or farm if spread could have occurred prior to the infestation being identified).

Particular care needs to be taken to ensure that soils and plant material are not moved into surrounding areas not showing symptoms of disease, as eggs or larvae can remain on seedlings and pupae can sometimes remain in the soil.

### 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(ies). These restraints may include restrictions or movement control for removal of plants, people, soil or contaminated equipment from an infected 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 pest. 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 infected premises and all suspected infected 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 (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 a detergent, a farm degreaser or a 1% 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

### 3.4.2 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 host growing properties in the pest quarantine area
- 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 presence of *Alternaria triticina*
- Surveying commercial nurseries selling at risk host plants (if applicable)
- Surveying other host growing properties and backyards

### 3.5.2 Survey regions

Establish survey regions around the surveillance priorities identified above. 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 2 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 2.*** *Phases to be covered in a survey plan*

| | |
|---|---|
| **Phase 1** | • Identify properties that fall within the buffer zone around the infested premise<br>• 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 infested 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 disease. Pathways to be considered are:<br>○ Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment<br>○ The producer and retailer of infected material if this is suspected to be the source of the outbreak<br>○ 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)<br>○ Movement of plant material and soil from controlled and restricted areas<br>○ Storm and rain events and the direction of prevailing winds that result in air-born dispersal of the pathogen during these weather events |
| **Phase 5** | • Surveillance of nurseries, gardens and public land where plants known to be hosts of pathogen 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. As a guide, the following activities should be carried out following the eradication of the pathogen:

- 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 a further 6 weeks and then on a monthly basis. Sentinel plants showing signs of the disease should be tested for confirmation then immediately removed and destroyed
- Surveys comprising of plant samples for testing for *A. triticina* to be undertaken for a minimum of two years after eradication has been achieved

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## 4.1 Websites

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

# 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 3) and diagnostic facilities (Table 4) for use in professional diagnosis and advisory services in the case of an incursion.

***Table 3.*** *Experts who can be contacted for professional diagnostic and advisory services*

| Expert | State | Details |
|---|---|---|
| No experts have been identified in Australia | | Dr Ian Pascoe (retired from DPI, Victoria) has expertise in morphological identification of *Alternaria* spp. |

***Table 4.*** *Diagnostic service facilities in Australia*

| Facility | State | Details |
|---|---|---|
| DPI Victoria Knoxfield Centre | Vic | 621 Burwood Highway<br>Knoxfield VIC 3684<br>Ph: (03) 9210 9222; Fax: (03) 9800 3521 |
| DPI Victoria Horsham Centre | Vic | Natimuk Rd<br>Horsham VIC 3400<br>Ph: (03) 5362 2111; Fax: (03) 5362 2187 |
| DPI New South Wales, Elizabeth Macarthur Agricultural Institute | NSW | Woodbridge Road<br>Menangle NSW 2568<br>PMB 8 Camden NSW 2570<br>Ph: (02) 4640 6327; Fax: (02) 4640 6428 |
| DPI New South Wales, Tamworth Agricultural Institute | NSW | 4 Marsden Park Road<br>Calala NSW 2340<br>Ph: (02) 6763 1100; Fax: (02) 6763 1222 |
| DPI New South Wales, Wagga Wagga Agricultural Institute | NSW | PMB Wagga Wagga<br>NSW 2650<br>Ph: (02) 6938 1999; Fax: (02) 6938 1809 |
| SARDI Plant Research Centre - Waite Main Building, Waite Research Precinct | SA | Hartley Grove<br>Urrbrae SA 5064<br>Ph: (08) 8303 9400; Fax: (08) 8303 9403 |

| Facility | State | Details |
|---|---|---|
| Grow Help Australia | QLD | Entomology Building<br>80 Meiers Road<br>Indooroopilly QLD 4068<br>Ph: (07) 3896 9668; Fax: (07) 3896 9446 |
| Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories | WA | 3 Baron-Hay Court<br>South Perth WA 6151<br>Ph: (08) 9368 3721; Fax: (08) 9474 2658 |

# Appendix 3. Communications strategy

A general Communications Strategy is provided in Appendix 6 of PLANTPLAN (2008, Version 1).

# Appendix 4. Market access impacts

Within the AQIS PHYTO database, the following countries require a specific statement regarding area freedom from *A. triticina* (October 2008). For the latest information, search the PHYTO database using an Advanced search "Search all text" for "*Alternaria triticina*".

| Country | Commodity | End use | Permit | Declaration required |
|---|---|---|---|---|
| New Zealand | *Triticum* spp. (wheat) seeds/grains | Sowing | Not required | "The seed has been sourced from a pest free area free from *Alternaria triticina*" or "The seed has been treated with a MAF approved treatment option for *Alternaria triticina*" |
| New Zealand | *Triticum* spp. (wheat) seeds/grains | Consumption | Required | Leaf blight (*Alternaria triticina*) is not known to occur in Australia |
| Brazil | *Triticum* spp. (wheat) seeds/grains | Sowing | Required | Samples inspected have been found free of Leaf blight (*Alternaria triticina*) |
| Brazil | *Triticum* spp. (wheat) seeds/grains | Consumption | Required | Samples inspected have been found free of Leaf blight (*Alternaria triticina*) |
| South Africa | *Triticum* spp. (wheat) seeds/grains | Sowing | Not required | Leaf blight (*Alternaria triticina*) is not known to occur in Australia |
| South Africa | *Triticum* spp. (wheat) seeds/grains | Consumption | Required | Leaf blight (*Alternaria triticina*) is not known to occur in Australia |