INDUSTRY BIOSECURITY PLAN
FOR THE GRAINS INDUSTRY

Threat Specific Contingency Plan

Leaf blotch of cereals

Bipolaris spicifera

(formally known as *Drechslera tetramera*)

Prepared by Dr Joe Kochman

and Plant Health Australia

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Plant Health AUSTRALIA

GRDC Grains Research & Development Corporation

CRC PLANTbiosecurity

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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 6215 7700
Fax:	+61 2 6260 4321
Email:	**admin@phau.com.au**
Website:	**www.planthealthaustralia.com.au**

1 Purpose of this Contingency Plan

This Contingency Plan was developed to provide background information on the pest biology and available control measures to assist with preparedness for an occurrence of significant leaf blotch of cereals (*Bipolaris spicifera*) in crops in Australia. During the development of this plan *B. spicifera* was found to be present in Australia and therefore a Response Plan would not be required.

2 Pest information/status

2.1 Pest details

Bipolaris spicifera (Bainier) Subram.

Other Names: *Cochliobolus spicifer* Nelson, *Bipolaris tetramera* (McKinney) Shoemaker, *Brachyclarium spiciferum* Bainer) Corbetta, *Drechslera spicifera* (Bainer) von Arx, *Drechslera tetramera* (McKinney) Shoemaker, *Brachycladium spiciferum* Brainier, *Curvularia spicifera* (Bainier) Boedijin, *Curvularia tetramera* (McKinney) Boedijin ex J.C. Gilman, *Helminthosporium spiciferum* (Bainier) Nicot, *Helminthosporium tetramera* McKinney, *Pseudocochliobolus spicifer* (R.R. Nelson) Tsuda, Ueyama & Nishih.

Common Names: Leaf blotch of cereals, spring dead spot of grasses, foot rot of wheat

2.1.1 General information

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

Bipolaris spp. are ubiquitous dematiaceous (dark-coloured) fungi that are commonly found in plant debris and soils in many regions throughout the world. Several species within this genus are pathogenic to both plants and animals, including *B. spicifera*. This fungal species is also commonly called *Bipolaris spicifera* and *Cochliobolus spicifer*.

In overseas countries; America, Asia, Africa and Europe, where this fungus occurs it is commonly found in seed batches of sorghum, wheat and barley (Fakhrunnisa *et al.,* 2006) where it can survive for extended periods of time. In addition, *B. spicifera* can survive within the soil (Patil *et al.,* 1974) and is among the most common pathogens found attached to combine harvesters in Egypt (Abdel-Hafez *et al.,* 1990). Together with the ability of *B. spicifera* to infect a wide range of species, these attributes allow maintenance of pathogen levels in the environment for lengthy periods of time.

The fungus has been recorded in Australia. There are 29 records of the fungus occurring in; NT, NSW, Qld and WA (Appendix 5, R Shivas pers. comm.). The first record was in 1974 at Ayr in Qld on *Medicago sativa* and most of the records are from Queensland. The fungus was found in NSW at Armidale (September 1975), Narrabri (May 1976) and Tamworth (March 1978) on undetermined species, sorghum and durum wheat respectively. The fungus was recorded in NT at Alice Springs (March 1989) on *Pennisetum clandestinum*. In WA the fungus was recorded at Watheroo (no date) on wheat and at Perth (August and September 1988) from humans and in July 1986 from a human patient (locality unknown). There do not appear to be any records of the fungus occurring in crop or

grass seed batches in Australia. Note: In all these records listed in three State collections the fungus is identified as *Cochliobolus spicifer*, the Teleomorph of *Bipolaris spicifera* (Alcorn, 1983).

B. spicifera utilises breakdown products of cellulose as an energy source, increasing sporulation rates when cultured on cellulose containing media (Pratt, 2006). During infection *B. spicifera* excrete a range of effector chemicals, including both phytotoxins and a plant growth promoter. The known chemicals are spiciferone A, B and C, spiciferinone (Nakajima *et al.,* 1993), cochliospicin (Nakajima *et al.,* 1997) and curvularin (Ghisalberti & Rowland, 1993), together with the plant-growth promoter molecule spicifernin (Nakajima *et al.,* 1993).

On detection of infection of *B. spicifera*, precautions should be undertaken to limit unprotected contact, as this fungus can also cause medical conditions in humans and other animals. These include fungal sinusitis, fungal peritonitis and "fungal balls" in airways. Fungal balls are a result of the growth of the fungi in airways following inhalation of spores.

This fungus has been recorded in four states and territories in Australia on a large range of hosts including; many grasses, wheat, sorghum, cotton, lucerne, *Araucaria cunninghamii* and three records from humans (all from WA) from 1974 to 2002. Hence, the fungus would appear to be endemic but has not caused serious crop losses or impacts to human health to date.

2.1.2 Life cycle

There appears to be little information specifically on the life cycle of *B. spicifera*, but the life cycles of all *Drechslera* and *Bipolaris* species are very similar. The fungus can survive as a saprophyte in trash of infected crops as spores and mycelium. The infected trash is a source of spores to infect new season crops. The spores can be spread rapidly over long distances to new crops as windblown spores or transferred within crops in rain splash, on vehicles, animals and clothing. Spores germinate in free water and infect leaves, either directly or through stomates, and then grow within the leaves to form blotches. As the leaves mature sporulation occurs within the blotches allowing a build up of inoculum for future crops. Heavy dews and moderate temperatures (18-25°C) favour the development of blotch diseases, caused by *Drechslera* and *Bipolaris* species, in several cereal crops (White, 1999; Frederiksen and Odvody, 2000).

2.2 Affected hosts

2.2.1 Host range

B. spicifera primarily infects the following species:

- Major hosts: *Cynodon dactylon* (Bermuda grass), *Triticum aestivum* (wheat), *Zoysia*
- Hosts where status is unknown: *Arachis hypogaea* (groundnut), *Capsicum* (peppers), *Eucalyptus tereticornis* (forest red gum), *Gossypium* (cotton), *Hordeum vulgare* (barley), *Luffa aegyptiaca* (loofah), *Lycopersicon esculentum* (tomato), *Musa* (banana), *Pennisetum glaucum* (pearl millet), *Piper betle* (betel pepper), *Populus* (poplars), *Saccharum officinarum* (sugarcane), *Sorghum bicolor* (sorghum), *Vicia faba* (broad bean), *Vigna aconitifolia* (moth beans).
- In Australia the fungus has been recorded on; *Sorghum vulgare*, *Triticum durum* (durum wheat), *Gossypium* (cotton), *Chloris virgata*, *Dactyloctenium radulans*, *Medicago sativa*, *Pennisetum clandestinum*, *Paspalidium caespitosum*, *Bothriochloa bladhii*, *Spinifex hirsutus*, *Triticum aestivum*, (wheat), *Araucaria cunninghamii* (Hoop pine), *Leptochloa divaricatissima*,

Oryza sativa (rice), *Sporobolus elongatus* and *Cynodon dactylon* (Shivas personal communication, Appendix 5)

2.2.2 Geographic distribution

B. spicifera is known to be present in the following countries (Figure 1):

- Asia: India, Iran, Iraq, Pakistan
- Europe: Greece, Hungary
- Africa: Egypt, Ghana
- South America: Argentina
- Australia (Note: Australia not included in Figure 1)

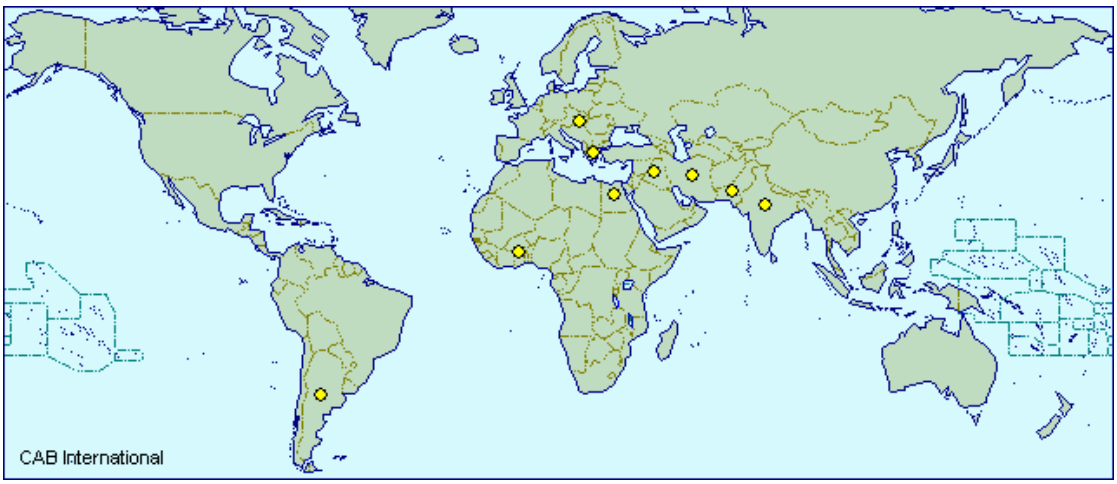

Figure 1. *Geographic distribution of B. spicifera reports. Yellow dots represent reports of B. spicifera detection. Figure obtained from* ***www.cabicompendium.org****.*

2.2.3 Symptoms

Leaf blotch of cereals can be present at all stages of plant development, however symptoms are mainly observed during the seedling stages of growth. Light yellow areas appear, particularly on the lower leaves. These areas increase in size over time and change from light yellow into dark brown, occasionally with a dark purple border. In some plant species the centre of the spots become ashy and shed off, leaving shot holes in the leaves.

Under wet weather conditions, the discolouration can spread from the leaves and into the stalks and crowns. When infection occurs in older plants the disease can result in defoliation.

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 blotch (then known by the scientific name *Drechslera tetramera*) on field peas and lentils 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 29 records for this pathogen under a range of synonyms (provided in Section 2.1) from a range of hosts. It is noted that certain pathotypes are already present and management practices are currently used. The pathogen has not caused serious crop losses to date.

2.3.1 Entry potential

Rating: High

The entry potential of new pathotypes of *B. specifera* is high for the following reasons:

- There are 29 records of the fungus in Australia in four states on a range of hosts (see Appendix 5). The *B. specifera* pathotypes have already entered the country and currently being managed and maintained
- *B. specifera* can be seed-borne and survive on infected plant residues for several years

2.3.2 Establishment potential

Rating: High

The fungus has been recorded in Australia on a wide range of hosts including: *Sorghum vulgare*, *Triticum durum* (durum wheat), *Gossypium* (cotton), *Chloris virgata*, *Dactyloctenium radulans*, *Medicago sativa*, *Pennisetum clandestinum*, *Paspalidium caespitosum*, *Bothriochloa bladhii*, *Spinifex hirsutus*, *Triticum aestivum*, (wheat), *Araucaria cunninghamii* (Hoop pine), *Leptochloa divaricatissima*, *Oryza sativa* (rice), *Sporobolus elongatus* and *Cynodon dactylon*. The first of the records held in State Plant Pathology Herbaria was in 1974 and the last in 2002. (Appendix 5, Shivas pers com).

2.3.3 Spread potential

Rating: High

The fungus has been recorded in four Australian states in widely separated areas on a wide range of hosts.

2.3.4 Economic impact

Rating: Low

The fungus has been present in Australia in some of the major grain growing areas for more than 30 years without causing any apparent crop losses. This situation may change if new varieties that are very susceptible to the fungus are developed.

2.3.5 Environmental impact

Rating: Low

The fungus has been present in Australia in some of the major grain growing areas for more than 30 years without any apparent affect on the environment.

2.3.6 Overall risk

Rating: Low

2.4 Diagnostic information

2.4.1 Diagnostic protocol

Identification of *B. spicifera* is a three stage process; growth morphology on nutrient plates, microscopic analysis of different growth stages, followed up by a PCR analysis for positive identification (Buzina *et al.,* 2001; Buzina *et al.,* 2003).

Surface sterilised plant tissue, or swabs of infected material, can be used to inoculate dextrose agar plates. Fungi from this genus will grow rapidly, with colonies reaching 3 to 9 mm in diameter after 7 days of growth at 25°C. Colony texture is velvety to woolly and is initially white to greyish brown and becomes green to black with raised greyish periphery as it matures. The underside of the colony is darkly pigmented and olive to black in colour. Additional information can be obtained through the analysis of inhibition zone diameters around antibiotic discs following 48 h incubation (Espinel-Ingroff *et al.,* 2007).

Further verification of fungal identity is carried our via microscopy analysis. The following features are characteristic of *B. spicifera*:

- Hyphae are septate with a brown pigmentation.
- Conidiophores (4.5-6 µm wide) are brown and unbranched. These bend at the points where each conidium arises leading to a zigzag appearance.
- Conidia (30-36 µm x 11-13.5 µm) are thick walled, three septate, fusoid to cylindrical in shape and light to dark brown in colour.

Confirmation of fungal identity requires PCR amplification of the internal transcribed spacer region of the ribosomal gene cluster with fungal-specific primers. Comparison of the sequence of the amplicon to those located on the GenBank database (**www.ncbi.nlm.nih.gov**; Benson *et al.,* 2008) will allow for species level identification of the pathogen. Positive sequence accession number is AY253918.

Specimens of most of the Australian records of this fungus are held at BRIP, the Queensland Primary Industries and Fisheries Plant Pathology Herbarium at Indooroopilly. Dr Roger Shivas is the current curator of BRIP (See Appendix 5).

2.5 Delimiting survey and epidemiology study

From the records this fungus has been recorded in four states and territories, found on a range of hosts, is considered to be endemic and has not caused significant crop losses to date. For these reasons it is unlikely that a delimiting survey and epidemiology study would be undertaken. However, it is possible a delimiting survey may be required if the fungi were detected in a new area.

The following sections (section 2.6.1 – 2.6.4) would only be required if a new, more virulent strain of this pathogen was suspected.

2.5.1 Sampling method

Once initial samples have been received and preliminary diagnosis made, follow up samples to confirm identification of the pathogen may 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 leaf blotch (*B. spicifera*) symptoms (i.e. light yellow areas appearing on the lower leaves of young seedlings and increasing over time to a dark brown colour with dark purple borders)

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.

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 or human health impacts 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 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.

All diagnoses of suspected exotic pathotypes should be undertaken according to the following parameters:

- The laboratory diagnostician has expertise in this form of diagnosis
- The results are confirmed by diagnosis in another recognised laboratory or by another diagnostician
- Where possible diagnosis is confirmed by a second method usually PCR

2.5.2 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

2.5.3 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 plant material can be blown into surrounding paddocks during harvesting and allow the pathogen to move considerable distances away from the infected crop
- 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 crops

2.5.4 Pest Free Area (PFA) guidelines

The establishment and maintenance of pest free areas (PFAs) would be a resource-intensive process, especially as the pathogen already occurs 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 2.5.1 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 for confirmation of fungal identity
- Seed sampling should be based on a minimum of 400 seeds (preferably 1000) as infection levels in seed may be low
- Surveys should also consider alternative host plants

2.6 Availability of control methods

2.6.1 General procedures for control

- Use fungicides as required (see possible fungicides in 2.6.6).
- Adopt best-practice farm hygiene procedures to retard the spread of the fungus between fields and adjacent farms.
- On-going surveillance of infected paddocks to ensure leaf blotch of cereals 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 blotch of cereals can be seed borne.

2.6.2 Control if small areas are affected

Spray with registered fungicide if required.

2.6.3 Control if large areas are affected

Spray with registered fungicide if required.

2.6.4 Cultural control

Rotate with non-host crops.

2.6.5 Host plant resistance

No information was found on strains of the fungus or on resistance in host plants. Should the disease occur in crops, trials will need to be conducted to determine the status and availability of crop plant resistance.

2.6.6 Chemical control

The use of selected fungicides on *B. spicifera* is effective at reducing disease levels and symptoms, but is not effective at eradication of the pathogen. The following fungicides are effective at reducing disease load:

- Thiophanate-methyl (Nan, 1995)
- Sulphuric acid/copper+zonc solution (Nan, 1995)
- Mancozeb (2.5 g/L)
- Copper oxychloride 3 g/L
- Thiobendazole
- Zineb
- Ziram
- Captan

Alternatives to chemically synthesised fungicides for *B. spicifera* include the use of allelopathic extracts from chickpea (*Cicer arietinum*; Bajwa *et al.,* 2006) and *Parthenium hysterophorus* (Bajwa *et al.,* 2003). Treatments with extracts from these plants are effective at reducing the fungal biomass by up to 41%.

2.6.7 Mechanical control

Burning stubble and trash where a crop was severely affected would reduce fungal biomass.

2.6.8 Biological control

Not available.

3 Course of action – eradication methods

As leaf blotch of cereals already occurs 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 are likely to reduce the likelihood of developing a technically feasible, cost beneficial eradication Response Plan.

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). If required the eradication process involves three main activities: surveillance, containment, and treatment and/or control measures.

3.1 Destruction strategy

3.1.1 Destruction protocols

Unless a new strain of the pathogen is detected very early and is readily distinguishable from established pathotypes, 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.

If eradication is considered, 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 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 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).

3.1.2 Decontamination protocols

Id decontamination procedures are required, 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 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

- 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

- 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, *B. spicifera* 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 *B. spicifera* 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 infected crops should be prevented as the dust created during harvesting can spread the disease to neighbouring areas
- Wind-borne inoculum can escape from *B. spicifera* 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 *B. spicifera* 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 infected property to other infected properties.

3.3.1 Destruction Zone

If destruction of hosts is considered, 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(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 infected area (and is therefore part of the Control Area) or may be adjacent to an infected 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.
- Plant material should be destroyed by high temperature incineration, autoclaving or deep burial (in a non-cropping area)

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 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 *B. spicifera* presence
- Surveying commercial grain traders that may have held infected seed
- Surveying commercial nurseries selling at risk host plants
- 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 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 nurseries, gardens and public land where plants known to be hosts of pest are being grown
Phase 6	• Agreed area freedom maintenance, post-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 pest:

- Establishment of sentinel plants at the site of infection (see Section 2.6.4).
- 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
- Surveys comprising of plant and soil sampling for use in testing for *B. spicifera* to be undertaken for a minimum of 3 years after eradication has been achieved

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4.1 Websites

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

Disease Management in Cotton (**www.jnkvv.nic.in/IPM%20Project/disease-cotton.html**)

National Centre of Biotechnology Information (**www.ncbi.nlm.nih.gov**)

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 Roger Shivas	Qld	Mycological Taxonomist and Curator BRIP, PIF, 80 Meiers Road, Indooroopilly, Qld 4068 Phone: (07) 3896-9340

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
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

Facility	State	Details
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 (2008, Version 1).

Appendix 4. Market access impacts

Within the AQIS PHYTO database, no countries appear to have a specific statement regarding area freedom from *B. spicifera* (May 2009). Latest information can be found within PHYTO, using an Advanced search "Search all text" for *Drechslera tetramera* or *Cochliobolus spicifer* or *Bipolaris tetramera* or *B. spicifera.*

Appendix 5. Records of the fungus in Australia

Records of *B. spicifera* in Australia as recorded in the Australian Plant Pest Database. Information was supplied by R. Shivas.

Record ID	Name	Host	Locality	Collector
BRIP 10939 a	*Cochliobolus spicifer* R.R. Nelson	*Triticum aestivum* L. (Poaceae)	Bunche, WA, Australia	
BRIP 10940 a	*Cochliobolus spicifer* R.R. Nelson	*Triticum aestivum* L. (Poaceae)	Watheroo, WA, Australia	
BRIP 10955 a	*Cochliobolus spicifer* R.R. Nelson	*Oryza sativa* L. (Poaceae)	Brisbane, QLD, Australia	Alcorn, J.L., 05 01 1970
BRIP 11166 a	*Cochliobolus spicifer* R.R. Nelson	Undetermined sp. ()	Armidale, NSW, Australia	Chick, B., 22 09 1975
BRIP 11169 a	*Cochliobolus spicifer* R.R. Nelson	Undetermined sp. ()	Taroom, QLD, Australia	Martyn, D., 22 09 1975
BRIP 11583 a	*Cochliobolus spicifer* R.R. Nelson	Undetermined sp. ()	Julia Creek, QLD, Australia	Connole, D., 08 1973
BRIP 11602 a	*Cochliobolus spicifer* R.R. Nelson	*Spinifex hirsutus* Labill. (Poaceae)	Stradbroke Island, QLD, Australia	Brooks, D., 18 01 1974
BRIP 11631 a	*Cochliobolus spicifer* R.R. Nelson	*Medicago sativa* L. (Fabaceae)	Ayr, QLD, Australia	Connole, M.D., 04 06 1974
BRIP 11720 a	*Cochliobolus spicifer* R.R. Nelson	Undetermined sp. ()	Amby, QLD, Australia	Clague, R., Connole, M.D., 18 05 1976
BRIP 12238 a	*Cochliobolus spicifer* R.R. Nelson	*Paspalidium caespitosum* C.E. Hubb (Poaceae)	Goondiwindi, QLD, Australia	Brouwer, Y., 25 04 1977
BRIP 12243 a	*Cochliobolus spicifer* R.R. Nelson	*Dactyloctenium radulans* (R.Br.) P. Beauv. (Poaceae)	Goondiwindi, QLD, Australia	Brouwer, Y., 25 04 1977
BRIP 12505 a	*Cochliobolus spicifer* R.R. Nelson	*Leptochloa divaricatissima* S.T.Blake (Poaceae)	Yelarbon, QLD, Australia	Alcorn, J.L., 11 05 1977
BRIP 12529 a	*Cochliobolus spicifer* R.R. Nelson	*Dactyloctenium radulans* (R.Br.) P. Beauv. (Poaceae)	Goondiwindi, QLD, Australia	Alcorn, J.L., 12 05 1977
BRIP 13218 a	*Cochliobolus spicifer* R.R. Nelson	Undetermined sp. ()	Torrens Creek, QLD, Australia	Johnson, S., 26 09 1980
BRIP 14195 a	*Cochliobolus spicifer* R.R. Nelson	*Araucaria cunninghamii* Aiton ex A.Cunn. (Araucariaceae)	Benarkin, QLD, Australia	Tierney, J.W., Diehm, W., 02 03 1983
BRIP 14434 a	*Cochliobolus spicifer* R.R. Nelson	*Triticum aestivum* L. (Poaceae)	Unknown, QLD, Australia	Mebalds, M., 25 06 1984

BRIP 15108 a	*Cochliobolus spicifer* R.R. Nelson	*Homo sapiens* ()	Unknown, WA, Australia	McAleer, R., 11 07 1986
BRIP 15696 b	*Cochliobolus spicifer* R.R. Nelson	*Bothriochloa bladhii* (Retz.) S.T. Blake (Poaceae)	Dalby, QLD, Australia	Carlsow, N.H., 23 06 1986
BRIP 16653 a	*Cochliobolus spicifer* R.R. Nelson	*Homo sapiens* (Linnaeus, 1758) (Hominidae)	Perth, WA, Australia	McAleer, R., 30 08 1988
BRIP 16655 a	*Cochliobolus spicifer* R.R. Nelson	*Homo sapiens* (Linnaeus, 1758) (Hominidae)	Perth, WA, Australia	McAleer, R., 05 09 1988
BRIP 17209 a	*Cochliobolus spicifer* R.R. Nelson	*Gossypium hirsutum* L. (Malvaceae)	Emerald, QLD, Australia	Harden, G., 20 02 1990
BRIP 18079 a	*Cochliobolus spicifer* R.R. Nelson	*Chloris virgata* Sw. (Poaceae)	Kilkivan, QLD, Australia	Gibbs, A.F., 04 06 1966
BRIP 18080 a	*Cochliobolus spicifer* R.R. Nelson	*Chloris virgata* Sw. (Poaceae)	Gatton, QLD, Australia	Gibbs, A.F., 07 11 1966
BRIP 18082 a	*Cochliobolus spicifer* R.R. Nelson	*Sporobolus elongatus* R.Br. (Poaceae)	Stanthorpe, QLD, Australia	Gibbs, A.F., 31 03 1967
BRIP 23120 e	*Cochliobolus spicifer* R.R. Nelson	*Dactyloctenium radulans* (R.Br.) P. Beauv. (Poaceae)	Musselbrook Reserve, QLD, Australia	Alcorn, J.L., 06 05 1995
BRIP 39191 a	*Cochliobolus spicifer* R.R. Nelson	*Cynodon dactylon* (L.) Pers. cv. *Plateau* (Poaceae)	Brisbane, QLD, Australia	Loch, D.S., 24 10 2002
DAR 26490 a	*Cochliobolus spicifer* Nelson	*Sorghum vulgare* Pers. (Poaceae)	Narrabri, NSW, Australia	Trimboli, D., May 1976
DAR 30669 a	*Cochliobolus spicifer* Nelson	*Triticum durum* Desf. cv. *Duramba* (Poaceae)	Tamworth, NSW, Australia	Southwell, R., Mar 1978
NTPPM 2010 a	*Cochliobolus spicifer* R.R. Nelson	*Pennisetum clandestinum* Chiov. (Poaceae)	Alice Springs, NT, Australia	Duff, J.D., 29 03 1989