INDUSTRY BIOSECURITY PLAN

FOR THE GRAINS INDUSTRY

Threat Specific Contingency Plan

Fusarium wilt (of chickpea, lentil & lupin)

Fusarium oxysporum f. sp. *ciceris*, *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *lupini*

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Plant Health AUSTRALIA

GRDC Grains Research & Development Corporation

CRC PLANTbiosecurity

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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 fusarium wilts of chickpea, lentil and lupin (*Fusarium oxysporum* f. sp. *ciceris, F. oxysporum* f. sp. *lentis* and *F. oxysporum* f. sp. *lupini*). 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

Scientific name	Other scientific names	Common names
F. oxysporum f. sp. *ciceris*	*F. lateritium* f. *ciceris* (Padwick) Erwin, *F. orthoceras* var. *ciceris* Padwick	Fusarium wilt of chickpea, wilt of chickpea, wilt of pigeon pea
F. oxysporum f. sp. *lentis*	*F. orthoceras* var. *lentis [anamorph]* Vasudeva & Srinov	Vascular wilt of lentil, wilt of lentil
F. oxysporum f. sp. *lupini*	*F. lupini*	Wilt of lupin

2.1.1 General information

Taxonomic position – Class: Ascomycetes; Subclass: Sordariomycetidae; Order: Hypocreales

Fusarium oxysporum is one of the most variable and highly dispersed species of *Fusarium*. The variability of *F. oxysporum* is reflected in the distribution and ecological activities of the species. Substantial populations are found in many native plant communities as well as in areas under cultivation, where they may be aggressive colonisers of the root cortex but are predominately non-pathogenic. Research activity has focused on the pathogenic strains occurring in agricultural soils.

The high level of host specificity of pathogenic strains in *F. oxysporum* led to the development of the *formae speciales* concept to enable better differentiation of these morphologically similar strains. Although host range is usually restricted to a few plant species, some *formae speciales* may have broader host ranges. As such, these groupings generally reflect phenotypic characteristics, and are not necessarily indicators of genetic relatedness, with one possible exception being *F. oxysporum* f. sp. *ciceris* (Jiménez-Gasco *et al.* 2002).

Vegetative compatibility, where two hyphae anastomose to form a stable heterokaryon, has been used to genetically distinguish and classify strains of *F. oxysporum*. However, genetic uniformity is not guaranteed for strains belonging to the same vegetative compatibility group (VCG) (Leslie and Summerell 2006). The implication here is that some VCGs may contain both pathogenic and non-pathogenic strains towards a common host.

2.1.1.1 Fusarium wilt of chickpea

Within the *Fusarium* genus, *F. oxysporum* is without doubt the most economically important species (Leslie and Summerell 2006). The anamorphic soil-borne fungus *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K.Satô is pathogenically associated with *Cicer* spp. (Nene *et al*. 1996), of which the high-value pulse crop, chickpea (*Cicer arietinum* L.) is the only cultivated species. Several diseases are known to limit worldwide production of chickpeas, of which *Fusarium oxysporum* f. sp. *ciceris* is one of the most important. Management of fusarium wilt has been primarily through development of resistant cultivars as part of an integrated management approach. However, the high pathogenic variability in populations of *F. oxysporum* f. sp. *ciceris* presents problems for sustainability of resistant cultivars.

Two pathotypes and eight races of the pathogen have been identified. The reliance on resistant cultivars for disease management of fusarium wilt therefore places significant importance on the confident and efficient identification of pathogenic races of *F. oxysporum* f. sp. *ciceris*. Using non-molecular methods, determination of the organism to the taxonomic level of *formae specialis* is costly in terms of time and resources. A PCR-based molecular assay has been developed that addresses these issues (Jiménez-Gasco & Jiménez-Díaz 2003).

While other *formae speciales* of *Fusarium oxysporum* are associated with plant groups in Australia, including important crops such as banana and cotton, *F. oxysporum* f. sp. *ciceris* is currently not known to occur in Australia.

2.1.1.2 Fusarium wilt of lentil

The Fusarium wilt that primarily infects lentils, *F. oxysporum* f.sp. *lentis* (Vasudeva & Srinavasan) Gordon, is present throughout many countries in Asia, Europe, Africa and America. *F. oxysporum* f. sp. *lentis* only infects the cultivated crop of lentil (*Lens culinaris* spp. *culinaris*) and wild vetch (*Vicia montbretii*). Transmission of the fungus occurs primarily through plant debris and soil contamination, where it infects the plant through the root system. There is also evidence of transmission through seeds (Erskine *et al.* 1990). Key factors in determining symptom expression and fungal growth rates are temperature and soil moisture (Dhingra *et al,* 1974; Saxena and Khare, 1988). Age of the plants also has an influence on the fungus with young seedlings producing root exudates containing glycine and phenylalanine which inhibited fungal spore germination (Claudius and Mehrotra, 1973).

There are no known physiological races within *F. oxysporum f.sp. lentis* (Bayaa *et al*. 1997, Belabid *et al*. 2004).

2.1.1.3 Fusarium wilt of lupin

A number of *F. oxysporum* f. sp. *lupini* races are present which are specific to different lupin species. Lamberts (1955) and Armstrong and Armstrong (1964) defined three races from European isolates. *Race 1* was pathogenic to *Lupinus luteus* and some cultivars of *L. albus* but non-pathogenic to *L. angustifolius*. *Race 2* was pathogenic to *L. luteus* and possibly all cultivars of *L. albus* but non-pathogenic to *L. angustifolius*. *Race 3* was pathogenic to *L angustifolius* and *L. albus* but non-pathogenic to *L. luteus*. All three races of *F. oxysporum* f. sp *lupini* in Europe were specific to lupins. Fusarium wilt pathogens from cowpea and cotton in the USA were pathogenic on some *L. luteus* cultivars (Armstrong and Armstrong 1964).

2.1.2 Life cycle

The life cycles of the Fusarium wilts of chickpea, lentil and lupin are essentially the same, varying only in the hosts they infect. Following infection of host roots, the fungus crosses the cortex and enters the xylem tissues. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. Seed infestation and infection is common. In chickpea, *F. oxysporum* f. sp. *ciceris* can be internally seed-borne and the pathogen is found as chlamydospore-like structures in the hilum region of the seed. The movement of infected seed plays an important role in the long distance dispersal and transmission of fusarium wilt diseases into new areas.

The root tips of healthy plants growing in contaminated soil are penetrated by the germ tube of spores or fungal mycelium. Entry is either direct, through wounds, or opportunistic at the point of formation of lateral roots. The mycelium takes an intercellular path through the cortex, and enters xylem vessels through the pits. The pathogen is primarily confined to the xylem vessels in which the mycelium branches and produces microconidia. The microconidia detach and are carried upward in the vascular system until movement is stopped, at which point they germinate and the mycelium penetrates the wall of the adjacent vessel. Lateral movement between vessels is through the pits.

The water economy of infected plants is eventually severely compromised by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant. The fungus then invades all tissues of the plant, to reach the surface where it sporulates profusely. Spores may then be dispersed by wind and water or movement of soil or plant debris.

F. oxysporum can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for more than 6 years (Singh *et al.* 2007). Chlamydospores can survive in soil either in dormant form or saprophytically for without a suitable host.

The disease is favoured by warm and dry soil conditions with an optimal temperature of 22-25°C.

2.2 Affected hosts

2.2.1 Host range

Each Fusarium wilt in general has a limited host range, as shown below:

Scientific name	Major host	Other hosts
F. oxysporum f. sp. *ciceris*	*Cicer arietinum* (chickpea)	*Cajanus cajan* (pigeon pea), *Lens culinaris* (lentil), *Pisum sativum* (pea)
F. oxysporum f. sp. *lentis*	*Lens culinaris* ssp. *culinaris* (lentil)	*Vicia montbretii* (vetch)
F. oxysporum f. sp. *lupini*	*Lupinus* (lupins)	

2.2.2 Geographic distribution

2.2.2.1 Fusarium wilt of chickpea

F. oxysporum f. sp. *ciceris* is present in countries of Europe, Asia, Africa and North America, as follows:

- Asia: Bangladesh, India, Myanmar, Nepal
- Europe: Italy, Mediterranean countries, Spain

- North America: USA

2.2.2.2 Fusarium wilt of lentil

F. oxysporum f. sp. *lentis* is present in countries of Europe, Asia, Africa and North and South America, as follows:

- Asia: India, Jordan, Nepal, Pakistan, Syria, Turkey
- Europe: Czechoslovakia (former), Russia, France, Hungary, Ukraine
- Africa: Egypt, Ethiopia, Morocco, Sudan, Tunisia
- North America: Canada
- South America: Argentina, Chile, Uruguay

2.2.2.3 Fusarium wilt of lupin

F. oxysporum f. sp. *lupini* is only present in countries within Africa and Europe, as follows:

- Africa: Egypt, South Africa
- Europe: Germany, Poland, The Netherlands, Belarus, Ukraine, Russia, Spain

2.2.3 Symptoms

2.2.3.1 Fusarium wilt of chickpea

Fusarium oxysporum f. sp. *ciceris* (races 1A, 2, 3, 4, 5 and 6) – Wilting pathotype.

Flaccidity of leaves and succulent shoots, followed by discoloration and chlorosis of leaves, desiccation and death; vascular (xylem) and pith tissues show discoloration, usually evident in cross sections of stem near the base.

Fusarium oxysporum f. sp. *ciceris* (races 0 and 1A/B) – Yellowing pathotype.

Progressive foliar yellowing from the base upwards; abscission of necrotic leaves; vascular (xylem) and pith tissues show discoloration.

Figure 1 shows typical distribution of chickpea plants infected with *F. oxysporum* f. sp. *ciceris* under field conditions. Careful examination of infected roots (Figure 2) can differentiate fusarium wilt from other diseases of seedlings and roots of chickpea. Wilt can be observed within 25 days of sowing into infected soil (Nene *et al.* 1978). Affected seedlings show drooping of the leaves and are a dull green colour. Seedlings may collapse and lie flat on the ground and, when uprooted, may show uneven shrinkage around the collar at the base of the stem. The roots do not show any external rotting and look apparently healthy. When split vertically from the collar region downward, such roots show a brown discolouration of the internal tissues. This combination of symptoms is unique to fusarium wilt. Infection by virus or Phytophthora root rot will produce some similar symptoms, but not identical.

Wilting may also occur in adult plants up until the reproductive and podding stage. Drooping of the petioles, rachis and leaflets in the upper part of the plant, together with the pale green colour of the foliage, are the most common symptoms. Often within 2 to 3 days the entire plant is affected (Haware *et al*., 1986). Lower leaves also become chlorotic. When uprooted before completely dried, affected plants show no external root discolouration. However, internal discolouration may be seen extending up towards the stem. Internal discolouration is due to infection of the xylem tissues of the root and

stem. Transverse sections of the infected root examined under the microscope show the presence of hyphae and spores of the fungus in the xylem. This is a diagnostic feature of Fusarium wilt. In certain chickpea cultivars typical symptoms may not develop. Instead, there is a yellowing and drying of the lower leaves, and a stunting of the plant. Roots will show internal discolouration. Figure 3 shows typical yellowing symptoms.

Figure 1: *Typical distribution of chickpea plants infected by F. oxysporum f. sp. ciceris under field conditions (Photo taken in Syria 2002).*

Figure 2: *Cross section of chickpea tap-root showing internal discolouration caused by F. oxysporum f. sp. ciceris infection.*

Figure 3: *An uprooted chickpea plant affected by Fusarium wilt, clearly showing typical yellowing symptoms.*

While the affected plant is alive the pathogen is confined to the vascular system and possibly a few surrounding cells. At plant death, the fungus moves to other tissues and sporulates at or near the plant surface. Plants grown from infected seed develop wilt symptoms faster than plants originating from clean seed.

2.2.3.2 Fusarium wilt of lentil

Symptoms can occur at both the seedling and adults stages of plant development. Disease effects both seedlings and adult stages and appear as patches in the field. Reports of the disease at the seedling stage orginate from India where, Fusarium infection is characterised by a sudden drooping of the leaves (more like wilting and damping off), followed by the leaves drying and the eventual death of the seedling. The root system will appear healthy, but with a reduced proliferation and nodulation rate. In most cases, there will be no discolouration of the vascular system. Other symptoms at the seedling stage include seed rot.

Generally symptoms of Fusarium wilt usually occur near or at the reproductive stages (flowering to pod-filling) of crop growth. Symptoms include the drooping and wilting of the uppermost leaflets,

which resembles water deficiency, stunting of plants, shrinking and curling of leaves that starts from the lower part of the plants and progressively moves up the stem. The remainder of the plant"s foliage or individual branches turns a dull green colour. The leaflets undergo closure and premature shedding from the plant. Plants finally become completely yellow and die. When plants are affected during the mid- to late-pod filling stages, seeds are often shrivelled.

Root symptoms include reduced growth with marked brown discolouration, tap root tips that are damaged and proliferation of secondary roots above the area of taproot injury. Often discolouration of vascular tissue may not be visible.

2.2.3.3 Fusarium wilt of lupin

Infection first becomes apparent in the vegetative stage causing leaves to darken. Obvious wilt symptoms begin at budding or flowering time when leaves dry out and rapidly defoliate. At this stage infected roots are almost symptomless except for a brown zone under the epidermis. Reddish-brown streaking of the vascular tissue is also sometimes visible on the upper stem and, under wet conditions, pink spore masses develop from the vascular streaks. This type of symptom is clearly visible in yellow lupin and in albus lupin. It is also expected to be characteristic in the blue lupin (*L. cosentinii*). However, this symptom is not diagnostic in narrow-leafed lupin as it occurs in healthy plants of many varieties. Infected plants eventually die.

Where the disease is severe, large areas of crop will be affected but may be more severe in drainage lines.

2.3 Entry, establishment and spread

2.3.1 Fusarium wilt of chickpea

Entry potential: Medium

The entry potential of *F. oxysporum* f. sp. *ciceris* is medium for the following reasons:

- *F. oxysporum* f. sp. *ciceris* is a seed-borne pest of chickpea
- Australia currently imports 150–200 tonnes of viable chickpea seed for human consumption annually. Current AQIS import conditions require that imported consignments be accompanied by a phytosanitary certificate declaring freedom from fusarium wilt. Despite this legislation, there is no complete guarantee that the pathogen cannot enter via infected seed or infested trash that may accompany the consignment
- Seed bought into Australia for sowing is required to be grown in an AQIS approved quarantine facility for one generation and inspected for disease symptoms before release
- Soil contaminated consignments also pose a risk of entry

Establishment potential: High

The establishment potential of *F. oxysporum* f. sp. *ciceris* in Australia is high for the following reasons:

- *Fusarium oxysporum* already occurs in Australia on other crop host species, demonstrating that suitable conditions do occur in Australia for the pathogen to survive
- Climatic conditions between countries such as Syria and India, where the disease already occurs, and areas of Australia are similar

- Chlamydospores of the pathogen can survive in the soil for many years in the absence of a host plant. The pathogen can also survive within infected plant material in the field
- Current commercial chickpea cultivars in Australia are highly susceptible to chickpea fusarium wilt
- *F. oxysporum* f. sp. *ciceris* can also be hosted by lentil and field pea (and show no symptoms) which are widely grown throughout the Australian cropping belt

Spread potential: High

The spread potential of *F. oxysporum* f. sp. *ciceris* in Australia is high for the following reasons:

- Spores can be splash dispersed, rain splash and moving water can carry chlamydospores and conidia short distances to surrounding plants and adjoining paddocks
- The pathogen can be transported over large distances in infected and infested grain and harvesting equipment and into new areas
- Grain infected by *F. oxysporum* f. sp. *ciceris* may not show external symptoms of infection
- Wind blown plant debris could spread the pathogen over moderate distances following harvest into adjacent paddocks

Economic impact: High

This disease has the potential to greatly downsize the chickpea industry in Australia in a similar manner to the ascochyta blight outbreak in 1998. An outbreak of Fusarium wilt of chickpea would result in a dramatic reduction in the area of production, due to increased costs of production making chickpea less competitive compared to other crops.

A substantial loss would also be incurred in the year of the outbreak. This not only includes lost production but also indirect impact on other business sectors such as other agricultural enterprises, storage, transport, manufacturing and wholesale trade. The losses would be similar to those incurred as a result of the outbreak of ascochyta blight in chickpeas in 1998 which has been calculated to have cost the Wimmera region in Victoria $62 million.

This disease causes serious yield losses in those countries where the pathogen is known to occur. Yield losses of up to 60% may occur under favourable conditions (Singh *et al.* 2007).

Environmental impact: Negligible

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

Social impact: Low

The reduction in the value of production and increased production costs would be expected to have low social impact.

Overall risk: High

2.3.2 Fusarium wilt of lentil

Entry potential: Medium

The entry potential of *F. oxysporum* f. sp. *lentis* is medium for the following reasons:

- *F. oxysporum* f. sp. *lentis* is a seed-borne pest of lentil
- Australia currently imports approximately 1200 tonne of lentil for human consumption annually. Current AQIS import conditions require that imported consignments be accompanied by a phytosanitary certificate if the seed is sourced from Canada, or moist heat treated to devitalise seed if sourced outside of Canada. Despite this legislation, there is no guarantee that the pathogen cannot enter via infected seed or infested trash or contaminated soil that may accompany the consignment.
- Seed bought into Australia for sowing is required to be grown in an AQIS approved quarantine facility for one generation and inspected for disease symptoms before release

Establishment potential: High

The establishment potential of *F. oxysporum* f. sp. *lentis* in Australia is high for the following reasons:

- *F. oxysporum* already occurs in Australia on other crop host species, demonstrating that suitable conditions do occur in Australia for the pathogen to establish and survive
- Climatic conditions between countries such as Syria and India, where the disease already occurs on lentil, and areas of Australia are similar
- Chlamydospores of the pathogen can survive in the soil for many years in the absence of a host plant. The pathogen can also survive within infected plant material in the field. This indicates that the pathogen is well adapted to survive adverse conditions.
- *F. oxysporum* f. sp. *lentis* can also be hosted by *Vicia* spp. which are widely grown throughout the Australian cropping belt

Spread potential: High

The spread potential of *F. oxysporum* f. sp. *lentis* in Australia is high for the following reasons:

- Spores can be splash dispersed. Rain splash and moving water can carry chlamydospores and conidia short distances to surrounding plants and adjoining paddocks
- The pathogen can be transported over large distances in infected and infested grain and harvesting equipment into new areas
- Grain infected by *F. oxysporum* f. sp. *lentis* may not show external symptoms of infection
- Windblown plant debris could spread the pathogen over moderate distances following harvest into adjacent paddocks

Economic impact: Medium

This disease has the potential to moderately downsize the lentil industry in Australia. The majority of commercial lentil varieties in Australia are susceptible to fusarium wilt. However, two current Australian lentil varieties („Northfield" and „Nipper") are resistant. An outbreak of fusarium wilt of lentil would result in a reduction in the area of production initially, until such time that growers could switch to using the resistant varieties. In the longer term, lentil production in regions where the pest is identified may be suspended as part of an eradication scheme. An outbreak of fusarium wilt would

fast track any lentil breeding lines that are identified with disease resistance but it may take several years for seed to be made available to growers.

Losses in lentil production would be incurred in the year of the outbreak. This not only includes lost production but also indirect impact on other business sectors such as other agricultural enterprises, storage, transport, manufacturing and wholesale trade. This pest causes serious yield losses in those countries where the pathogen is known to occur. Complete crop losses may occur under favourable conditions of a warm, dry spring. In addition, screening of lentil breeding lines for resistance to fusarium wilt found that yield losses can range from 25-95% depending on the variety (Baraimer and Izquierdo 1977).

Environmental impact: Negligible

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

Social impact: Low

The reduction in the value of production and increased production costs would be expected to have low social impact.

Overall risk: Medium

2.3.3 Fusarium wilt of lupin

Entry potential: Rating = Low

The entry potential of *F. oxysporum* f. sp. *lupini* is low for the following reasons:

- *F. oxysporum* f. sp. *lupini* is a seed-borne pest of lupin
- The entry potential of *F. oxysporum* f. sp. *lupini* is low while quarantine restrictions on the import of soil, lupin plant material and seed are in place
- Lupin seed is generally not imported into Australia for human consumption. Lupin seed is most likely to be imported as seed for sowing. Lupin seed bought into Australia for sowing is required to be grown in an AQIS approved quarantine facility for one generation and inspected for disease symptoms before release.

Establishment potential: High

The establishment potential of *F. oxysporum* f. sp. *lupini* in Australia is high for the following reasons:

- *Fusarium oxysporum* already occurs in Australia on other crop host species, demonstrating that suitable conditions do occur in Australia for the pathogen to survive
- Climatic conditions between countries such as Egypt and South Africa, where the pest already occurs, and areas of Australia are similar
- Chlamydospores of the pathogen can survive in the soil for many years in the absence of a host plant. The pathogen can also survive within infected plant material in the field
- Current commercial lupin cultivars in Australia are highly susceptible to lupin fusarium wilt

Spread potential: High

The spread potential of *F. oxysporum* f. sp. *lupini* in Australia is high for the following reasons:

- Spores can be splash dispersed. Rain splash and moving water can carry chlamydospores and conidia short distances to surrounding plants and adjoining paddocks
- The pathogen can be transported over large distances in infected and infested grain and harvesting equipment into new areas
- Grain infected by *F. oxysporum* f. sp. *lupini* may not show external symptoms of infection
- Windblown plant debris could spread the pathogen over moderate distances following harvest into adjacent paddocks

Economic impact: High

The economic impact of *F. oxysporum* f. sp. *lupini* is likely to be high for the following reasons:

- Disease screening of current Australian commercial lupin cultivars has found all cultivars to be highly susceptible to *F. oxysporum* f. sp. *lupini*
- The incidence of the disease increased in Germany after first being reported in 1906 and by the 1930s production of yellow lupin was seriously affected
- Fusarium wilt of lupins remains an important disease in Eastern Europe
- The disease has been devastating in Germany, Poland and the former USSR. Growing of lupins in these countries was not possible until the development of resistant varieties of lupin were bred

Environmental impact: Negligible

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

Social impact: Low

The reduction in the value of production and increased production costs would be expected to have low social impact.

Overall risk: Medium

2.4 Diagnostic information

2.4.1 Diagnostic protocol

There are several methods of identifying fusarium wilt on chickpea, lentil and lupin. These include:

- Identification of the *F. oxysporum* species based on morphology. This will only indicate if *F. oxysporum* is present, and not the *formae speciales*
- Identification based on PCR testing. This is currently only available for *F. oxysporum* f. sp. *ciceris*
- Identification based on host species. This method will identify *formae speciales*

2.4.1.1 Identification based on morphology

Identification based on morphology can be done with direct visual examination of the pathogen recovered from infected plants. This type of identification is not entirely conclusive and should be done in conjunction with other identification methods. Identification is based on spore size, ornamentation and colour. It will not indicate host specificity. Spores have to be mounted onto slides and inspected using a microscope.

F. oxysporum can defined to some degree by morphological criteria, including the shape of micro- and macroconidia, the structure of the microconidiophore (false heads on short phialides formed on the hyphae), and the formation of chlamydospores (Nelson ***et al***. 1983).

Microconidia are abundant, aseptate, reniform to oval, produced in false heads on short monophialide conidiophores. They range from 5-12µm x 2.-3.5µm. Macroconidia are rare. Chlamydospores are profuse in culture and are formed singly or in pairs.

On Carnation Leaf-piece Agar (CLA), macroconidia of *F. oxysporum* are formed in pale orange sporodochia borne from monophialides on branched conidiophores, or sometimes from monophialides on hyphae. The macroconidia are short to medium in length, falcate to almost straight, thin-walled and usually 3-septate. The basal cell is notched or foot-shaped, and the apical cell slightly hooked in some isolates. Microconidia are formed abundantly in false heads on short monophialides (Figure 4). They may be oval, elliptical or reniform, and are usually without septa (Figure 5).

In most isolates, chlamydospores are formed abundantly and rapidly (2-4 weeks), but formation may be slow (4-6 weeks) or not at all in some isolates. Chlamydospores are usually formed singly or in pairs, but may be found in clusters or small chains. They may be either terminal or intercalary, and are most obvious in hyphae on the agar surface, although they may appear in submerged hyphae.

Figure 4: *Microconidia in false heads on short monophialides on hyphae of F. oxysporum*

Figure 5: *Microconidia of F. oxysporum*

On Potato Dextrose Agar (PDA), colony morphology varies widely. Colony growth on PDA is rapid with white aerial mycelium that may become slightly tinted with orange. The undersurface may be colourless to faintly green or blue. Colony colour is largely dependent on incubation conditions. *F. oxysporum* usually produces a pale to dark violet or dark magenta pigment in the agar, but some isolates produce no pigment at all. Mycelia may be floccose; sparse or abundant; and range in colour from white to pale violet. Abundant pale orange or pale violet macroconidia are produced in a central spore mass in some isolates. Small pale brown, blue to blue-black or violet sclerotia may be produced abundantly in some isolates. The appearance of some isolates is influenced by mutation to the pionnotal form or to a flat „wet" mycelial colony with a yellow to orange appearance on PDA.

Although there is considerable variation in these structures, *F. oxysporum* can be distinguished from *F. solani*, which forms microconidia on false heads on long monophialides, and from *F. subglutinans*, which forms microconidia on polyphialides and does not form chlamydospores.

Identification to infraspecific level for *F. oxysporum* using morphology is problematic due to the diversity of non-pathogenic or saprophytic strains in soil, and the difficulties in distinguishing these from pathogenic strains based on morphological or cultural criteria. The inherent cost in terms of time and resources to characterise isolates to subspecies levels and further into pathogenic races, has necessitated the development of molecular methods to achieve satisfactory determinations. A PCR-based assay has been developed (Jiménez-Gasco and Jiménez-Díaz 2003) that will identify all races of the *F. oxysporum* f. sp. *ciceris* pathogen. The assay involves isolation the organism from infected plant material, DNA extraction and amplification using a specific primer in a PCR.

2.4.1.2 Fusarium wilt of chickpea

Diagnosis of Fusarium wilt of chickpea is a two-stage process. Firstly, a preliminary microscopic examination is undertaken to determine whether disease symptoms and pathogen morphology are consistent with chickpea Fusarium wilt or an endemic disease, such as phoma, rhizoctonia root rot or phytophthora root rot. The primary diagnostic test, a PCR, is undertaken to determine if the pathogen

is *F. oxysporum* f. sp. *ciceris*. An experienced plant pathologist should perform the preliminary examination. The primary test requires sample processing in a specialised laboratory capable of molecular techniques.

The diagnosis of Fusarium wilt of chickpea would need to be performed quickly and accurately.

Further details can be found in the *F. oxysporum* f. sp. *ciceris* Diagnostic Protocol.

2.4.1.3 Fusarium wilt of lentil

Field diagnosis should be done in connection with paddock cropping history. A recent lentil production history could indicate potential wilt problems. Suspect stunted and wilted plants should be carefully removed from the soil so that the roots can be checked for reduced growth without external fungal growth. External fungal growth indicates the presence of other diseases such as rhizoctonia or phoma. Lower stems should be split to check for vascular discolouration. Although vascular discolouration is not always symptomatic of fusarium wilt the presence of discolouration would confirm the disease. Culturing of infected plant tissue in the laboratory should be done with caution because of the possible presence of other saprophytic *Fusarium* spp. that appears similar to *F. oxysporum* f. sp. *lentis*. A pathogenicity test on lentil is necessary to confirm *F. oxysporum* f. sp. *lentis*.

2.4.1.4 Fusarium wilt of lupin

Initial diagnosis of Fusarium wilt of lupin is through vascular decolouration in yellow lupins, however this is not possible for narrow leafed lupin, as the vascular tissue of narrow leafed lupin is naturally discoloured. During this investigation vascular tissue can also be plated out onto Fusarium selective media.

2.4.2 Identification based on host species

Identification of the *formae speciales* of *F. oxysporum* can be done through pathogenicity testing on a set of differential species. This would indicate the host specificity of the isolate and hence whether the isolate is exotic. The differential hosts used for each test will clearly vary depending on the target *formae speciales*.

A seedling test may also be performed on suspected infected seed. A method is suggested by Haware *et al*. (1986) for the identification of fusarium wilt of chickpea. The same basic test could be applied to identification of seed-borne fusarium wilt in lentil and lupin.

2.4.2.1 Fusarium oxysporum f. sp. ciceris

A suggested set of differential plant species would include:

- Lentil (*Lens culinaris*) – widely grown commercial variety, but not „Nipper" or „Northfield" which are resistant
- Field pea (*Pisum sativum*) – variety selection is not important as all are susceptible
- Chickpea (*Cicer arietinum*) – variety selection not important
- Faba bean (*Vicia faba*) – variety selection not important
- Vetch (*Vicia sativa*) - variety selection not important

- Lupin (*Lupinus albus*) - variety selection not important

A positive test result for *F. oxysporum* f. sp*. ciceris* would be a susceptible reaction on lentil, chickpea and field pea only. Faba bean, vetch and lupin would be resistant.

2.4.2.2 Fusarium oxysporum f. sp. lentis

A suggested set of differential plant species would include:

- Lentil (*Lens culinaris*) – widely grown commercial variety, but not „Nipper" or „Northfield" which are resistant
- Lentil (*Lens culinaris*) – resistant variety „Nipper" or „Northfield"
- Field pea (*Pisum sativum*) – variety selection is not important
- Chickpea (*Cicer arietinum*) – variety selection not important
- Faba bean (*Vicia faba*) – variety selection not important
- Vetch (*Vicia sativa*) - variety selection not important
- Lupin (*Lupinus albus*) - variety selection not important

A positive test result for *F. oxysporum* f. sp. *lentis* would be a susceptible reaction on lentil and vetch only. A resistant reaction would be expected on lentil varieties „Nipper" or „Northfield". Field pea, faba bean, chickpea and lupin would be resistant.

2.4.2.3 Fusarium oxysporum f. sp. lupini

A suggested set of differential plant species would include:

- Lentil (*Lens culinaris*) - widely grown commercial variety, but not „Nipper" or „Northfield" which are resistant
- Field pea (*Pisum sativum*) – variety selection is not important
- Chickpea (*Cicer arietinum*) – variety selection not important
- Faba bean (*Vicia faba*) – variety selection not important
- Vetch (*Vicia sativa*) - variety selection not important
- Lupin (*Lupinus albus*) - variety selection not important
- Lupin (*Lupinus angustifolius*) - variety selection not important
- Lupin (*Lupinus luteus*) - variety selection not important

A positive test result for *Fusarium oxysporum* f. sp*. lupini* would be a susceptible reaction on lupin species only, all other pulse species would be resistant. The type of reaction on *Lupinus* spp. would indicate which race/s are present.

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 and 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 of poor growth. The normal procedure is to collect symptomatic plants and to test them to confirm the presence of *F. oxysporum* f. sp*. ciceris, F. oxysporum* f. sp. *lentis* and *F. oxysporum* f. sp*. lupini* depending on the crop species being sampled. 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. It should be noted that spread of *F. oxysporum* f. sp*. ciceris, F. oxysporum* f. sp. *lentis* and *F. oxysporum* f. sp*. lupini* is restricted to the movement of contaminated soil, movement of water from contaminated areas, and movement of contaminated plant material including seed.

Containment of the pathogen will depend on:

- The density of host crops grown in the affected area
- The prevailing weather conditions, especially rainfall and movement of surface water
- The initial surveys being completed quickly

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

2.6.1 Sampling method

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

General protocols for collecting and dispatching samples are available within PLANTPLAN, Appendix 3 (Plant Health Australia 2008).

2.6.1.1 Number of specimens to be collected

- Plant sampling should be based on at least 100 plants taken at random per crop
- Seed sampling should be based on a minimum of 400 seeds, but preferably 1000 seeds should be tested.

Samples should be initially collected over a representative area of the infected crop to determine the disease distribution. The disease may appear as „hotspots" or patches within the crop or may have developed along fence lines or drainage lines. Depending on the stage of infection the symptoms may appear as:

- Small patches of yellowing (dying) plants scattered throughout the crop
- Larger patches of yellowing and dying throughout the crop
- Patches of yellowing and dying plants along fence or drainage lines from adjacent paddocks

All foliage can become infected by *F. oxysporum*; this includes leaves, stems and pods. 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.

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 or surface water from adjacent paddocks or originated from contaminated machinery or human movement.

Field inspections should include a transect or track through a field that allows representative sampling of the entire field with, on average, one inspection site of 10 m^2 of plants per hectare. Plants should be assessed for yellowing, stunting, wilt and plant death. Stems should be broken or sliced to reveal vascular discolouration in case plants are not showing external symptoms.

Aerial inspections would also be useful as Fusarium wilts often occur in patches or along rows in fields and these patches are usually visible from the air (Kochman, pers. obs.). Remote sensing and infra-red technologies have also been used to identify bare areas in cotton fields. Field inspections of these areas are needed to confirm that they are caused (in fact) by a *Fusarium* pathogen.

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.

Field soil from affected crops may also be collected as this will also harbour *F. oxysporum*. Soil may be used for pathogenicity testing of host pulse crop species to identify *formae specilaes* or put into long term storage as a reference.

2.6.1.2 How to collect samples

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. Once removed from the field, fresh plant samples can deteriorate and become contaminated by other mould fungi and bacteria. 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.

2.6.1.3 How to preserve plant samples

Long term storage of fungal isolates can occur and 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 nearby 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 results are confirmed by diagnosis in another recognised laboratory or by another diagnostician
- Where possible diagnosis is confirmed by a second method

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 PLANTPLAN, Appendix 3 (Plant Health Australia 2008).

2.6.2 Epidemiological study

There are many factors that affect the development of fusarium wilt diseases in fields. These include: the presence of virulent strains in the soil, susceptibility of the crop varieties, soil type, soil fertility, climatic conditions, irrigated or non-irrigated crops and interactions with other soil borne micro-organisms. Inoculum densities in the soil are also important as disease symptoms may not be apparent when there are low levels of the pathogenic strains in the soil (Davis ***et. al***. 2006).

Symptoms may occur in a small number of plants or in large areas in any part of the field. Lange and McLaren (2002) found that, in a targeted survey of 12 canola fields in Manitoba, Canada, the disease was not seen in the random 100-plant samples collected from three of the 12 fields even though it was present in other areas of these fields highlighting the irregular nature of occurrence of the disease. Hence, as well as random transect surveys, aerial surveys would also be useful in identifying bare, dead, stunted or wilted areas in fields which would then need to be checked to confirm or deny the presence of the pathogen.

The disease can occur at any stage of plant growth, particularly when crops are stressed. Factors such as, high plant populations, improper cultivation, other soil-borne pathogens and various herbicides are all known to induce injury of young roots, causing plant stress. These can aggravate fusarium wilt damage. Early infections can kill seedlings soon after emergence, leaving bare areas in the crop. Later infection can cause wilting, yellowing and death of adult plants.

The effect of fusarium wilt is most apparent during blossoming and early pod set when the plant and its productivity are more sensitive to stress.

The number of infected plants within a crop will depend on the source and amount of primary inoculum available and whether environmental conditions have been favourable for the pathogen 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 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 host pulse crops to the initial infected crop, both in the current growing season and previous season. Alternate host crops should also be considered as these crops can also harbour the pathogen in some instances. This will include the growers own pulse crops and pulse crops on neighbouring properties
- Machinery or vehicle movements into the infected crop. Especially the possible movement of contaminated soil
- 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

The pathogen is disseminated within and among fields by the movement of contaminated soil by wind, irrigation water, overland flood flows, on machinery and workers" clothing. As an example, fusarium wilt in cotton was likely to have been spread from the Darling Downs to the St George area of Queensland (a distance of some 400 km) in flood flows because the first fields affected in the St George area were irrigated from flood flows. Pathogenic strains of *F. oxysporum* are able to survive for long periods in soil and infected crop residues, either as a saprophyte or as chlamydospores. Hence the importance of minimising overland flood flows over any areas identified as affected.

Seed production must not occur in any affected areas to minimise the possibility of seed transmission of the pathogen to new areas.

Spread may occur in the following ways:

- Movement of infected or infested seed. The pathogen has the potential to be transmitted in infected seed. However, seedlots can also become infested with contaminated soil and small infected plant fragments which may transmit the pathogen
- Mechanical transmission through movement on contaminated vehicles, machinery and humans
- Small fragments of pod, stem or leaf tissue carrying the pathogen can be blown into surrounding paddocks during harvesting and allow movement over considerable distances away from the infected crop
- Fungal spores that adhere to clothing, machinery or animals can be carried large distances into other crops
- Run-off off surface water that may carry spores or contaminated soil into waterways or surrounding paddocks

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 2.6.1 for points to consider in the design).
- Plant sampling should be based on at least 100 plants taken at random per crop.
- Seed sampling should be based on a minimum of 400 seeds, but preferably 1000 seeds should be tested. The author has been unable to find indicative figures for the level of seed transmission of *F. oxysporum*.
- 5 kg of soil sampled per paddock. Enough soil to perform a small scale host species experiment if required (see Section 2.4.2).
- Survey around irrigation systems or waterways that may have transported chlamydospores.
- Surveys should also consider alternative host crops.
- The use of aerial inspection or remote sensing may be possible, with suspect patches inspected and sampled to confirm or deny the presence of fusarium wilt.

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

Once introduced and established, pathogenic strains of *F. oxysporum* can survive in soil for extended periods, even in the absence of crop hosts, making eradication a long term process. Hence containment procedures to retard the spread of the pathogen are required and suitable procedures have been developed by the Australian cotton industry following the discovery of fusarium wilt in cotton in 1993 (Kochman 1995). Procedures developed to retard the spread of cotton fusarium wilt can be directly applied to retard the spread of fusarium wilt in chickpea, lentil and lupin. They are described in the cotton IDM guidelines (Allen et al. updated 2003) and other publications available on the Cotton Catchment Communities website (**www.cottoncrc.org.au**) under the publications, disease and microbiology section.

2.7.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas. Contaminated soil can easily be transported through mechanical means (movement of vehicles and machinery, human)
- Cease irrigating affected crop areas and use bunding to divert overland flood flows and surface water run-off 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 infected crops and seed lots should be undertaken. Infected crops should be destroyed by burning and ploughing. Any infected seed lots should be incinerated or buried deeply (in a non-cropping area)
- On-going surveillance of infected paddocks to ensure fusarium wilt is eradicated will be necessary for at least 5 years
- Ensure that seed production does not take place on affected farms and do not use seed from these farms to plant next crop as fusarium wilt can be seed borne

2.7.2 Control if small areas are affected

Pull out the affected plants, as well as healthy plants 5-10 metres into the area surrounding the patch and burn them in the patch. Particular care must be taken to minimize the transfer of infected soil from the area. Raking and burning the whole field at this stage is NOT an option as this procedure is likely to spread the pathogen over the field.

2.7.3 Control if large areas are affected

Kill any surviving plants in the area, preferably with herbicides and ploughed in. Once the dead plants have broken down, sow an alternative crop such as a cereal or grass pasture to prevent erosion. All equipment used on the site should be thoroughly cleaned down, with products such as a farm degreaser or a 1% bleach solution and washed down with a pressure cleaner on the affected farm. The clean down procedure should be carried out on hard standing or preferably a designated wash-down area to avoid mud being recollected from the affected site onto the machine. A wash-down pad design and operational procedures are described in the cotton IDM package.

2.7.4 Cultural control

Fusarium wilt can be managed through the use of disease prevention strategies, such as rotation with non-host crop species, control of volunteer pulse crop plants in cereal crops, and control of annual weeds in crop borders and headlands. Soil amendment with organic matter, such as wheat or barley straw, has been found to enhance antagonism by other soil micro-organisms. Delayed sowing can reduce disease incidence, but late sowing can dramatically reduce yield potential and its effect on disease development can differ over locations and seasons (Kannaiyan and Nene 1975). However, as the causal pathogen can survive in soils for long periods of time, there still can be some residual disease inoculum even with good management practices (Lange and McLaren 2002), hence eradication can become a long term process. Since fusarium wilt of chickpea, lentil and lupin are seed borne, the use of clean seed from disease-free crops is very important.

2.7.5 Host plant resistance

Development of plant lines resistant to Fusarium wilts is the most effective approach to the management or eradication of the disease. Breeding of resistant lines and identification of DNA markers for resistance to fusarium wilt has been achieved in chickpea (e.g. Sharma *et al.* 2005), lentil (e.g. Bayaa *et al.* 1998; Eujayl *et al.* 1998) and lupin (e.g. Lamberts 1955). It is important to note that in some cases, resistant plant varieties are only suitable for use against certain fusarium wilt races (Jiménez-Gasco *et al.* 2004). Currently all Australian commercial varieties of lupin and chickpea are

susceptible to fusarium wilt. However, there are resistant chickpea breeding lines available in Australia that can be utilised in the event of a fusarium wilt incursion and the Australian Lupin Breeding Program screens Australian developed breeding lines for fusarium wilt resistance in Poland. The Lentil Breeding Program in Australia appears to be the most advanced with several current commercial lentil lines available with good levels of disease resistance. This breeding program is also actively screening advanced breeding lines in Syria annually for evaluation in the fusarium wilt sick plot.

2.7.6 Chemical control

Seed applied and foliar fungicides, crop rotation, offer some control of fusarium wilt, but are generally not as effective as the use of resistant varieties. In lentil, where fusarium wilt can appear at the seedling stage, the use of fungicide seed dressings can be effective in reducing disease severity. Lentil seed treatment with thiram + pentachloronitrobenzene or thiram + carboxin reduced the incidence of the disease (Bayaa and Erskine 1998). For chickpea and lupin obtaining useful levels of fusarium wilt control with seed-applied fungicides can be difficult because the disease appears to be most aggressive late in the growing season, long after seed-applied fungicides can reasonably be considered effective. However, seed- treatments may reduce losses by eliminating or reducing seed-borne inoculum sources. Foliar fungicides tend to be expensive, and must be applied as protective sprays well before symptoms become apparent (Lange and McLaren 2002) in addition to the technical difficulty of incorporating chemicals into the soil.

Fumigants provide control of fusarium wilt (but not eradication), but are not cost effective for routine use in chickpea, lentil and lupin.

2.7.7 Mechanical control

Deep ploughing over summer and removal of infected trash can reduce inoculum levels of fusarium wilt of chickpea (Haware 1998). Solarisation of soil by covering the soil with transparent polythene sheeting for 6-8 weeks during the summer months has been shown to effectively control fusarium wilt of chickpea and improve plant growth and yield (Chauhan *et al*. 1988). However, this method of control is not practical for broad-acre farming systems.

2.7.8 Biological control

No commercial biological control agents that directly attack Fusarium pathogens are currently available. However, potential biological agents have been identified for control of these fusarium wilt diseases. In India, *Trichoderma viride*, *Streptomyces gougeroti* and several bacterial species were found to be antagonistic to *F. oxysporum* f. sp. *lentis* (Mehrotra and Claudius 1972). Similarly, *Trichoderma harzianum* and *Trichoderma koningii* have shown antibiosis and mycoparasitism (Mukhopadhyay *et al*. 1989). Most recently El-Hassan and Gowen (2006) have investigated the use of *Bacillus subtilis.* A comparison of formulations found that use of either talc or glucose significantly decreased disease severity and enhanced plant growth promoting activity by increasing root length in lentil. Wahid (2006) found *T. pseudokoningii* and *B. subtilis* to inhibit the growth of *F. oxysporum* f. sp. *lupini* when applied to seed and sown in the field in Egypt. Similar results have been found for the control of *F. oxysporum* f. sp. *ciceris*, with *Trichoderma* spp. reducing plant mortality when applied to seed and sown in the field (Kumar *et al.* 2006). Numerous other micro-organisms have been reported as potential bio-control agents of *F. oxysporum* f. sp. *ciceris* including *Rhizobium* (Arfaoui *et al.* 2005,

2006), *Bacillus* spp. (Jamali *et al.* 2005, Landa *et al.* 2004) and *Pesudomonas* spp. (Anjaiah *et al*. 2003)

Biological modification of soil microflora has proved successful in reducing disease severity (Mazzola, 2002). Enrichment of soils with the beneficial mycorrhiza fungi (Goswami *et al.* 2007) or *Rhizobium leguminosarum* bacteria (Essalmani and Lahlou, 2003) may provide an effective management strategy against pathogenic fungal species. In addition, prior inoculation of chickpea with non-pathogenic isolates of *F. oxysporum* significantly reduced disease incidence and severity (Hervás *et al.* 1995).

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. Chlamydospores may persist within infected soil for up to eight years or longer.
- The paddock may be cropped with cereals or oilseed crops for several years following the incursion and selective herbicides used to ensure the area remains free of host pulse crop plants such as lentil, chickpea or lupin and other potential host plants.
- All vehicles and farm machinery that enter the infected field should be thoroughly washed, preferably using a detergent such as Decon 90 or farm degreaser.
- Any infected plant material or soil removed from the site should be incinerated, autoclaved or buried deeply (in a non-cropping area).
- If the pathogen is detected very early it could be eradicated. It is able to produce spores that are dispersed only short distances via rainsplash, infected soil and infected plant trash including seed. The pathogen is also capable of being transported over long distances via the movement of infected seed and contaminated vehicles and machinery.
- 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 area. 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
- Determine the extent of infection through survey and seed trace back
- Priority of eradication/decontamination of infected host material
- Inform all groups in the industry
- Stop the movement of any seed that may be infected with *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *lupini*, or *F. oxysporum* f. sp. *ciceris*

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)
- As the fungus can be mechanically transmitted, killed crops should be ploughed in, to prevent the spread of dead, infected plant material.
- Seed harvested from infected plants and any infected soil or plant material removed from the paddock 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
- Infested paddocks should remain free of host plants for at least five to eight years

3.1.5 Disposal issues

- Particular care must be taken to minimize the transfer of infected soil or plant material from the area as Fusarium wilt can survive in soil for long periods of time, even in the absence of plant hosts
- 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 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.

3.2.2 Movement control for people, plant material and machinery

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, to and from affected farms, must be controlled to ensure that infected soil or plant debris is not moved off-farm on clothing, footwear, vehicles or machinery.

Examples of movement controls include:

- 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 recollected from the affected site onto the machine (refer to Section 3.1.2 for details)
- 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

Destruction Zone may be defined as contiguous areas associated with the same management practices as the infected area (i.e. the entire trial, paddock or farm if spread could have occurred prior to the infection being identified).

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.

The movement of air-borne inoculum to adjacent host crops will be 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. Spores of the rust fungi will be readily disturbed with human and machinery movement, and movement should be minimised.

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

Decontaminant practices are aimed at eliminating the pathogen 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 pathogen 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, farm degreaser or a 1% (available chlorine) bleach solution in a designated wash down area as described in 3.1.2
- Plant material should be destroyed using herbicide and burnt if possible. If burning cannot be carried out then plant material should be buried by cultivation. This reduces the chance of disease spread from old infected plant material. Only recommended materials are to be used when conducting decontamination procedures, and should be applied according to the product label

3.4.2 Decontamination if pest is identified in small or large areas

Destruction of plant material by herbicide is recommended. The infected area would need to be monitored for a few years for self sown plants which should be tested for Fusarium wilt and then destroyed.

3.4.3 General safety precautions

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

3.5 Surveillance and tracing

3.5.1 Surveillance

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

Initial surveillance priorities include the following:

- Surveying all properties in the quarantine area with known hosts
- Surveying all properties identified in trace-back and trace-forward 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 lentil and field pea rust
- Surveying commercial grain traders that may have held contaminated seed

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 infested 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 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: ○ Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment ○ The producer and retailer of infected material if this is suspected to be the source of the outbreak ○ 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) ○ Movement of plant material and soil from controlled and restricted areas ○ 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

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 plant sampling for and testing for fusarium wilt to be undertaken for a minimum of 12 months after eradication has been achieved

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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
Joop van Leur	NSW	NSW Department of Primary Industries Tamworth Centre for Crop Improvement 4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
James Cunnington	Vic	DPI Victoria - Knoxfield 621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9436; Fax: (03) 9800 3521
Brett Summerell	NSW	Royal Botanic Gardens - Sydney Mrs Macquarie Road Sydney NSW 2000 Ph: (02) 9231 8113; Fax: (02) 9241 1135

Table 3. *Diagnostic service facilities in Australia*

Facility	State	Details
The University of Melbourne BioMarka	Vic	Faculty of Land and Food Resources The University of Melbourne VIC 3010 Ph: (03) 8344 9753; Fax: (03) 8344 9753
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

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

Appendix 3. Communications strategy

A general Communications Strategy is provided in Appendix 6 of PLANTPLAN (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 *F. oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *lentis*, or *F. oxysporum* f. sp. *lupini* (August 2008). Should *F. oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *lentis*, or *F. oxysporum* f. sp. *lupini* be detected or become established in Australia, some countries may require specific declaration. Latest information can be found within PHYTO, using an Advanced search "Search all text" for *"Fusarium oxysporum* f. sp. *ciceris*" or "*Fusarium oxysporum* f. sp. *lentis*" or "*Fusarium oxysporum* f. sp. *lupini*".