

**INDUSTRY BIOSECURITY PLAN
FOR THE GRAINS INDUSTRY**

Threat Specific Contingency Plan

**Verticillium wilt of canola
*Verticillium longisporum***

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and Plant Health Australia
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1 Purpose and background 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 Verticillium wilt of canola (*Verticillium longisporum*). It provides guidelines and options for steps to be undertaken and considered when developing a Response Plan for incursion of Verticillium wilt of canola. 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.

The information for this plan has been primarily obtained from documents as cited in the reference section. Information on background, life cycle, host range, distribution and symptoms of Verticillium wilt of canola are given, with the emphasis of this document on the management options in the event of an incursion in Australia.

2 Australian Grains Industry

The Australian Grains Industry is primarily situated in a narrow crescent running through the mainland states, known as the grain belt. This area stretches in a curve from central Queensland, through New South Wales, Victoria and southern South Australia. In Western Australia, the grain belt covers the south-west corner of the state.

The grains industry is the largest plant industry and grain crops are grown in all states and territories. The gross value of grains and oilseeds in 2006/07 was \$5.3 billion, compared to the five year average for gross value of grains and oilseeds from 2002/03 – 2006/07 of \$7.4 billion per annum (ABS data). The grains industry consists of 25 leviabile crops; however, Verticillium wilt of canola is predominantly a threat to canola and other *Brassica* crops. The average annual production of canola (*Brassica napus*) from 2003-2008 was approximately 1.2 million tonnes with an average 900 000 hectares sown annually (Australian Oilseeds Federation 2008).

Due to Australia's relatively small population and domestic demand, export markets are essential for the viability of Australian grain farms. Australia exports the majority of the canola crop to countries including Japan, China, Pakistan and Bangladesh. With this reliance on exports, maintaining our current plant health status through appropriate biosecurity measures is of utmost importance in retaining access to these markets.

3 Eradication or containment decision matrix

The decision to eradicate should be based on the potential economic impact of host damage resulting from Verticillium wilt of canola, the cost of eradication and on technical feasibility. Eradication costs must factor in long term surveys to prove the success of the eradication program. As the spores can survive in the soil for up to 10 years (Heale and Karapapa 1999) a minimum of 10 years with no detection of Verticillium wilt of canola may be necessary before pest free status can be declared.

No specific eradication matrix has been determined for Verticillium wilt of canola, however the general decision process as outlined in Figure 1 should be followed in determining if an incursion of this pest

will be eradicated or managed/contained. The final decision between eradication and management will be made through the National Management Group.

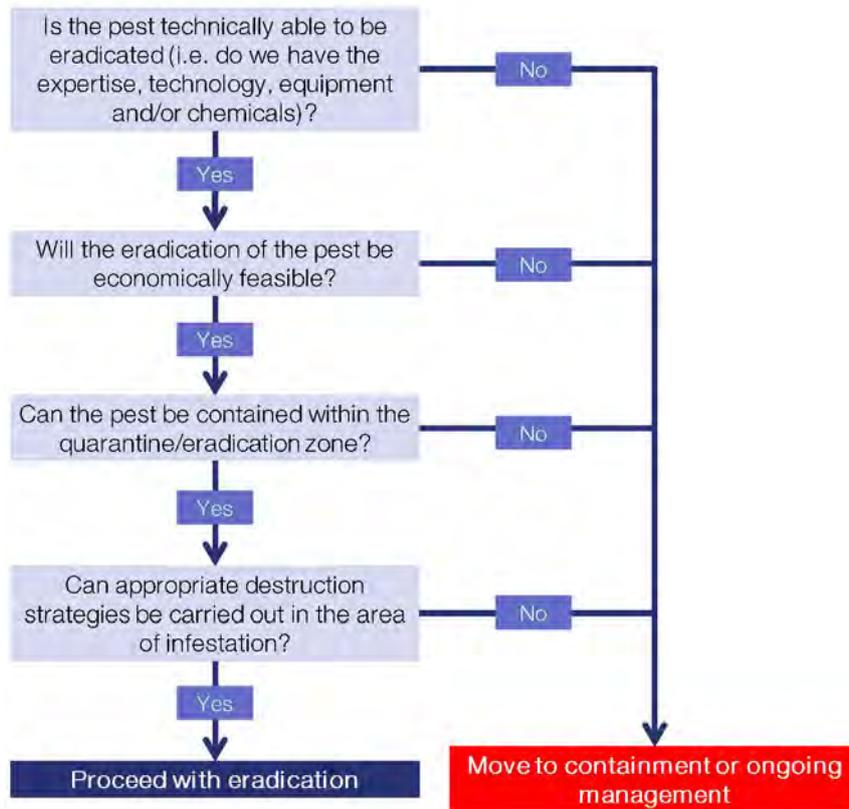


Figure 1. Decision outline for the response to an exotic pest incursion

4 Pest information/status

4.1 Pest details

Scientific name	Other scientific names	Common names
<i>Verticillium longisporum</i>	<i>Verticillium ovatum</i> , <i>V. dahliae sensu</i> , <i>V. albo-atrum</i> var. <i>medium</i> , <i>V. trachiephilum</i> , <i>V. dahliae</i> var. <i>longisporum</i>	Verticillium wilt

Taxonomic position – Phylum: Ascomycota; Class: Ascomycetes; Order: Pezizales; Family: Sarcosomataceae

4.1.1 General information

Verticillium longisporum is a soil-borne fungus that causes vascular wilting in *Brassica* crops with symptoms similar to those caused by *Fusarium oxysporum* (Fusarium wilts). *V. longisporum* is an important pathogen in canola crops across Europe and Russia (Heale and Karapapa, 1999, Portenko, 2000, Dixelius *et al.*, 2005) and *Brassica* vegetable crops in the USA (Koike *et al.*, 1994). Control of the disease is particularly problematic due to unavailability of approved fungicides and resistant lines at present, and the persistence of viable spores in soil for 10-15 years (Heale and Karapapa, 1999).

V. longisporum was originally classified as a variant of *V. dahliae* with unusually long conidia (Ingram, 1968) but has since been recognised as a separate species based on morphological and physiological differences (Karapapa *et al.*, 1997). These differing features have been summarised by Zeise and von Tiedemann (2001). *V. longisporum* is also distinctive in pathogenicity in that it is mainly a pathogen of *Brassica* plants but has limited pathogenicity to other plants that are susceptible to *V. dahliae* (Zeise and von Tiedemann, 2002).

V. dahliae (Verticillium wilts) affects over 160 plant species is already present and widespread in Australia but as yet *V. longisporum* has not been reported.

4.1.2 Life cycle

V. longisporum is a root-inhabiting fungus (Domsch *et al.*, 1980) which can survive in soil as microsclerotia which germinate in response to root exudates. This step does not seem to be species specific, with exudates from non-host plants also stimulating germination of *V. longisporum* (Mol and Vanriessen, 1995). Microsclerotia can survive in the soil for up to 10-15 years (Heale and Karapapa, 1999).

V. longisporum grows and multiplies in the plant's vascular system, blocking the xylem vessels with hyphal growth. In addition, products resulting from the activity of pectic enzymes restrict vascular flow and disrupt the host's metabolism by altering the permeability of the host's membranes. All of these factors contribute to a blockage of the xylem system (CSIRO, 1996).

The life cycle of the fungus has not been extensively studied but is thought to be very similar to that of the widely studied *V. dahliae*. The life cycle can be summarised as follows:

- Microsclerotia in soil germinate in response to root exudates (Mol and Vanriessen, 1995)
- The hyphae or germinating conidia produced by microsclerotia penetrate the cortex of young roots (usually root hairs or lateral roots) of host plants and the fungus grows into the stele. The onset of flowering is a critical phase for *V. longisporum* to colonise canola plants (Zhou *et al.*, 2006)
- In the xylem vessels the pathogen spreads by mycelial growth, and also by the production of conidia which become transported in the transpiration stream; in this way the pathogen rapidly becomes systemic in susceptible crops. Conidia can become trapped in vessel end walls, where they germinate and re-colonise the tissue, before more conidia are produced to continue colonisation of upstream vessels (Beckman, 1987)
- The major disease effects are believed to result from displacement of vessels and the production of toxins
- During senescence, the fungus moves out of the xylem vessels and into the surrounding non-vascular tissues where microsclerotia form in the senescing diseased tissues (Mol and Scholte, 1995). The microsclerotia can remain viable in soil for up to a decade under suitable conditions. They survive over a range of soil moisture and temperature conditions, but lose

viability most rapidly in wet, warm soil (Green, 1980, CPC, 1999). Both bacteria and fungi can attack and degrade the microsclerotia in soil, reducing survival periods (Baard *et al.* 1981).

4.2 Affected hosts

4.2.1 Host range

Brassica crops are the predominant hosts of *V. longisporum* (Zeise and von Tiedemann, 2002), with canola (*Brassica napus*) considered the primary host.

Other hosts of *V. longisporum* include cauliflower (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), broccoli (*B. oleracea* var. *botrytis*), brussels sprouts (*B. oleracea* var. *gemmifera*) (CPC, 1999), turnip (*B. oleracea* var. *gongylodes*; *B. rapa* subsp. *rapa* plus other varieties) (Horiuchi *et al.*, 1990) and other *Brassica* sp.. Horseradish (*Armoracia rusticana*) is also affected (Stark, 1961). Radish (*Raphanus sativas*) and wild radish (*R. raphanistrum*) may be important weed hosts, although further research is needed.

More recent research suggests that non-*Brassica* crops such as wheat, pea and oats can be infected by *V. longisporum* (though only 5-20 % of samples had formed Microsclerotia compared to >80 % in canola) (Johansson *et al.*, 2006) and can thus act as reservoirs for inoculum.

4.2.2 Geographic distribution

V. longisporum has a limited distribution, with reports of pathogen presence arising from Europe and Russia on canola (Heale and Karapapa, 1999, Portenko, 2000, Dixelius *et al.*, 2005), and in Japan and the USA on other *Brassica* sp. (CPC, 1999, Subbarao *et al.*, 1995). Other species of *Verticillium* (including *V. dahliae*) are more widely distributed, including present in Australia.

4.2.3 Symptoms

Because the disease often appears later in the season and plants suffer from water stress (due to blockage of the vascular system), early symptoms can be ambiguous and resemble symptoms of *Fusarium oxysporum* (Bailey *et al.* 2003). The disease becomes more serious in the presence of plant-pathogenic nematodes or if drought and cropping stress are experienced (Jacobsen *et al.*, 1979, Martin *et al.*, 1982, Tchatchoua and Sikora, 1983, Grontoft and Jonasson, 1992).

The most extreme symptom of *Verticillium* wilt of canola is irreversible wilting of the whole plant followed by plant death. In some cases wilting may only affect some shoots or leaves and occasionally wilting may not occur at all. Specific symptoms include premature leaf fall, stem necrosis, a reduction in stem diameter and stunting (Grontoft and Jonasson, 1992). Chlorosis of branches and leaves and bronzing of stems may also be observed before protruding microsclerotia give the stems a blackened appearance (Dixelius *et al.*, 2005). *Verticillium* induced discolouration of stems often coincides with discolouration due to blackleg (*Leptosphaeria maculans*) making symptom differentiation difficult (Kuusk *et al.*, 2002).

Symptoms listed by plant part:

- Whole plant: plant dead; dieback; early senescence, stunting
- Leaves: chlorosis, necrotic areas; abnormal colours; abnormal leaf fall; wilting
- Stems: stunting or rosetting; dieback; internal discolouration, blackening as microsclerotia appear

4.3 Entry, establishment and spread

4.3.1 Entry potential

Rating: High

V. longisporum is transmitted by seed. Australia currently imports stock feed of plant origins which may contain canola seed from a wide range of countries including those where *V. longisporum* is known to occur. Even though the stock feed would be imported under appropriate AQIS conditions, there is no guarantee that the pathogen cannot enter via infected seed or infected trash that may accompany the consignment. In addition, *Brassica* spp. seed is also approved to be imported as seed for sowing. AQIS requirements are that consignments are visually inspected for contaminants (mainly soil and foreign seeds) before the consignment is released. There is always the possibility that seeds infected by *V. longisporum* may not be detected as part of this process.

4.3.2 Establishment potential

Rating: High

V. dahliae is already widely distributed in Australia and there is no biological reason to suggest that *V. longisporum* would not react similarly under Australian climatic conditions. Microsclerotia can survive in the soil for up to 10-15 years (Heale and Karapapa, 1999) in the absence of a host plant and therefore disinfection of soil would be difficult. *Verticillium dahliae* already occurs in Australia on other crop host species, demonstrating that suitable conditions do occur in Australia for this genus to survive.

4.3.3 Spread potential

Rating: High

The high spread potential is largely due to the fact that the pathogen is disseminated by infected seed. Infected grain may not show external symptoms of the disease. Microsclerotia in soil and plant debris can also be transported via contaminated equipment or vehicles. Microsclerotia can also be splash dispersed; rain splash and moving water can carry them short distances to surrounding plants and adjoining paddocks. Microsclerotia and microsclerotia in plant debris can also be windblown into adjoining paddocks. The pathogen can be transported over large distances in infected and infested grain and harvesting equipment and into new areas.

4.3.4 Economic impact

Rating: High

European studies have reported up to 70% yield losses in individual rape (canola) plants due to premature ripening. One study that has investigated the influence of Verticillium wilt and *Heterodera schachtii* (beet cyst eelworm/sugarbeet nematode) on canola in greenhouse experiments estimated seed yield reductions of up to 30% when canola plants were infected by the pathogen alone and reductions of up to 53% when the pathogen was found in combination with *H. schachtii* infestations (Grontoft and Jonasson, 1992). Seed reduction due to the combined effect of *V. longisporum* and *H. schachtii* is pertinent to Australia as *H. schachtii* is already widespread in Australian grain growing regions (McLeod *et al.*, 1994). In addition, there are no resistant canola cultivars available which means that growers would be unable to grow canola or readily find a replacement oilseed crop. Persistence of microsclerotia in the soil means that canola could not be grown in contaminated paddocks for many years and that alternate crops would have to be grown.

4.3.5 Environmental impact

Rating: Low to Medium

V. longisporum has a limited host range and pathogenicity (see Section 4.2.1); however, it is predicted to impact on native species in the Brassicaceae family.

4.3.6 Overall risk

Rating: High

4.4 Diagnostic information

4.4.1 Diagnostic protocol

The pathogen will usually grow from vascular tissue excised from suspect plants under moist conditions at 22°C, producing typical conidiophores. The most reliable diagnosis is to excise vascular tissue from stems or petioles, aseptically transfer to a culture medium and incubate the tissues at approximately 22°C. Prune yeast lactose agar is recommended (Talboys, 1960), on which conidiophores and dark, resting mycelium are readily produced. Other media used routinely in studies on *V. dahliae* include Potato-carrot-dextrose agar (PCDA) and minimal agar medium with L-sorbose (MM) (Puhalla and Hummel, 1983). In their study sanguinarine medium (SM) consisted of commercial Potato dextrose agar (PDA) amended with 500 ppm sanguinarine nitrate before autoclaving. Horiuchi *et al.*, (1990) recommend using diluted Hall and Ly's medium for identifying *V. longisporum* (referred to as *V. dahliae* var. *longisporum*: Group D in their study).

V. longisporum can be distinguished from *V. dahliae* by morphological and physiological differences or through molecular analysis.

Morphological and physiological diagnosis

Key morphological and physiological features that differ between *V. longisporum* and *V. dahliae* from Karapapa *et al.* (1997) and Zeise and von Tiedemann (2001) as adapted by Tappe (2008) are shown in Table 1.

Table 1. Morphological and physiological characteristics of isolates of *V. longisporum* and *V. dahliae*¹

	<i>V. longisporum</i>	<i>V. dahliae</i>
Shape of conidia	Elongate	Small, spherical
Conidial length [µm]	7.0 – 7.9	3.2 – 5.5
Colony colour (solid medium)	Black	White
Dark mycelium (liquid medium)	Present	Absent
Microsclerotia	Long	Rounded
Polyphenol oxidase activity	None	None
Pigment secretion (liquid medium)	None	Weak
Sporulation rate (shake cultures)	Low	High

Molecular diagnosis

The DNA content of *V. longisporum* is generally twice that of *V. dahliae* and small subunit ribosomal RNA (SSU-rRNA) of *V. longisporum* contains a 839 bp intron at position 1165 that is not present in *V. dahliae* (Karapapa and Typas, 2001). Steventon *et al.* (2002) developed a PCR-based analysis for confirmation of *V. longisporum* which has been used successfully in later studies (Zhou *et al.*, 2006).

¹ Sourced from Karapapa *et al.* (1997) and Zeise and von Tiedemann (2001) as adapted by Tappe (2008)

5 Pest management

5.1 Response checklist

The following checklist (Table 2) provides a summary of generic requirements to be identified and implemented within a Response Plan.

Table 2. Checklist of requirements to be identified in a Response Plan

Checklist item	Further information
Destruction methods for plant material, soil and disposable items	Section 6.1.1, 6.1.2
Disposal procedures	Section 6.1.5
Quarantine restrictions and movement controls	Section 6.2
Decontamination and property cleanup procedures	Section 6.4
Diagnostic protocols and laboratories	Section 4.4, 8.2
Trace back and trace forward procedures	Section 6.5
Protocols for delimiting, intensive and ongoing surveillance	Section 5.2
Zoning	Section 6.3
Reporting and communication strategy	See PLANTPLAN 2011

A range of specifically designed procedures for the emergency response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia, 2011).

5.2 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 *V. longisporum*. If confirmed, plants taken at random from the same crop should be tested to enable an estimate to be made of the disease incidence. Surrounding crops would then be surveyed. The extent of the survey beyond the initial infected crop should be guided by the test results from surrounding crops.

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

5.2.1 Sampling method

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

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

All plants should be assessed for the presence of the *V. longisporum* symptoms. Depending on the stage of infection the symptoms may appear as (see Section 4.2.3 for full details):

- Wilting of the whole plant
- Premature leaf fall
- Reduction in stem diameter and presence of necrosis
- Stunting of plant growth
- Chlorosis of leaves

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, 2011).

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 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.

While *V. longisporum* is a soil-borne fungus, following plant infection it is present in the vascular tissues of the stem and leaves. Collection of stem sections will provide the best material for diagnostic testing for the pathogen. 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 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 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

Seed sampling should be based on a minimum of 400 seeds but preferably 1000 seeds should be tested.

5.2.1.1 HOW TO COLLECT AND PLANT 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. For appropriate labelling and packaging procedures for suspect emergency plant pests consult PLANTPLAN (Plant Health Australia, 2010).

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°C freezer and stored for an indefinite period without damaging fungal DNA.

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°C, which can be used to extract DNA.

5.2.2 Epidemiological study

There are many factors that affect the development of 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 as shown for Fusarium wilt in cotton (Davis *et. al.* 2006).

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 Brassica 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 Brassica crops and Brassica 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

5.2.3 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 and microsclerotia which may transmit the pathogen
- Mechanical transmission through movement on contaminated vehicles, machinery and humans
- Small fragments of pod, stem or leaf tissue, or microsclerotia carrying the pathogen can be blown into surrounding paddocks during harvesting and allow movement over considerable distances away from the infected crop
- Run-off from surface water may carry microsclerotia or contaminated soil into waterways or surrounding paddocks
- Transportation of microsclerotia in contaminated soil adhering to footwear, animals or machinery within the infected crop.

5.2.4 Pest Free Area (PFA) guidelines

The establishment and maintenance of pest free areas (PFAs) would be a resource-intensive process. Prior to development of a PFA 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 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).

Points to consider are:

- Design of a statistical delimiting field survey for symptoms on host plants (See 5.2.1 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

5.3 Availability of control methods

Once introduced and established, these pathogens 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.

5.3.1 General procedures for control

- Keep traffic out of affected areas and minimise movement in adjacent areas.
- Stop irrigating affected (irrigated crops) areas and use bunding to divert overland flood flows around them (both irrigated and dryland crops).
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms.
- After surveys are completed, destruction of the infected crops and seed lots should be undertaken. Infected crops should be sprayed with an appropriate herbicide to kill the crop/s and destroyed by burning, followed by ploughing. Any infected seed lots should be incinerated or buried deeply (in a non-cropping area).
- On-going surveillance of infected paddocks to ensure *V. longisporum* is eradicated for 10 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 *Verticillium longisporum* can be seed borne.

5.3.2 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.

5.3.3 Control if large areas are affected

Kill any surviving plants in the area, preferably with herbicides, burn and plough 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.

5.3.4 Cultural control

There is no known sexual reproduction stage for *V. longisporum*, meaning that it is considered to be a single life cycle disease. Therefore, measures aimed at reducing the initial inoculum levels in the soil should be effective control measures for this disease (Pennypacker, 1989). Approaches that are effective at reducing the inoculum levels in the soil are:

- Crop rotation strategies, using crops that are not susceptible to *V. longisporum*. The spores of *V. longisporum* can survive in the soil for up to 10-15 years. Therefore susceptible crops cannot be grown in infected fields for over a decade using this approach. Alternatively a pasture of non-host species could be sown and monitored for pathogen presence.
- Destroying all infected plants at the site as well as any susceptible weeds.
- Incorporation of ryegrass, broccoli, cauliflower, maize and Indian mustard residues have been shown to reduce microsclerotia inoculum in soils to some degree but results were variable and depended on soil type (Debode *et al.* 2005).
- Activities that increase soil biological activity should be encouraged as a means of accelerating breakdown of microsclerotia, this may involve sowing of non-host species and strategic tillage practices

5.3.5 Host plant resistance

The use of resistant crop cultivars is the most effective method for control of Verticillium wilt of canola and the lack of a sexual reproduction stage and a low gene flow means that *V. longisporum* is considered a low-risk pathogen when considering resistance breeding (McDonald & Linde, 2002). Extensive screening failed to find significant sources of resistance within *B. napus* germplasm (Happstadius *et al.*, 2003); however, promising sources of resistance have been found in *B. rapa* and *B. oleracea* germplasm (Happstadius *et al.*, 2003, Dixelius *et al.* 2005) which are currently being used in breeding programs in Europe to create canola cultivars with multiple sources of resistance to *V. longisporum* (Rygulla *et al.* 2007).

5.3.6 Chemical control

During the early stages of the disease, the fungal pathogen infects the host plant and grows and propagates inside the vascular tissue (Schnathorst, 1981). Therefore, the application of fungicides is ineffective as they cannot reach the pathogen without killing the plant.

Reduction of the inoculum in the soil is the alternative chemical control approach; however, the effectiveness of this approach is limited to the ability of the chemical to reach all inoculum in the soil. The use of elemental sulphur as an antifungal agent (Cooper & Williams, 2004) can be used in combination with other control methods to reduce the effect of this disease on the crop. Debode *et al.* (2005) reported that chemicals such as methyl bromide have been used to kill microsclerotia in soils, but no data on efficacy of such treatments were provided nor references given.

5.3.7 Mechanical control

Mechanical control such as ploughing the entire crop into the soil will limit spread of the disease to other paddocks/farms within infected plant material, but because spores reside in soil for up to 10-15 years mechanical control is limited in effectiveness for eradication of the disease. Mechanical control may increase biological activity in the soil which may increase the rate of breakdown of microsclerotia. Activities that increase soil biological activity should be encouraged as a means of accelerating breakdown of microsclerotia, this may involve sowing of non-host species and strategic tillage practices.

5.3.8 Biological control

The use of biological control against *V. longisporum* is not common in the field, however significant progress has been made in identifying bacterial strains that have a potential to effectively control fungal levels. Early work had identified *Talaromyces flavus* (Nagtzaam, 1998), *Bacillus subtilise*, *Pseudomonas fluorescens* and *Stenotrophomonas maltophilia* (Berg *et al.*, 1994) as effective biocontrol agents against *V. longisporum*. *S. maltophilia* was found to be the most effective of this group (Berg *et al.*, 1994). Two newer bacterial lines identified from an antagonistic rhizosphere to phytopathogenic fungal growth are *Serratia plymuthica* C48 (Berg *et al.*, 1999) and *Paenibacillus alvei* K165 (Tjamos *et al.*, 2004). *S. plymuthica* C48 reduces *V. longisporum* growth through the excretion of a chitinase enzyme (Berg *et al.*, 1999), while *P. alvei* K165 does not directly affect the pathogenic fungi (Tjamos *et al.*, 2005). The mechanism of *P. alvei* K165 effect on *V. longisporum* is the induction of a systemic host response through the host plant (Tjamos *et al.*, 2005).

Many of the microbes present on the seeds of canola naturally have a strong direct inhibition on *V. longisporum* (about 50% of microbes from resistant varieties of oilseed rape; Granér, *et al.*, 2003). These bacteria may be partially responsible for the host-plant resistance seen in many cultivars. Alternatively, the addition of these microbes to infected sites, or as a pre-emptive measure, may provide a potential method for biological control.

6 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.

6.1 Destruction strategy

6.1.1 Destruction protocols

- Infected crops should be destroyed by burning and ploughing. This will prevent aerial dispersal of the pathogen via infected crop residues.
- 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.
- 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.
- Fungal microsclerotia are likely to be carried into nearby crops and microsclerotia can survive in the soil for 10-15 years. The pathogen is also likely to be transported over long distances via the movement of infested seed and contaminated vehicles and machinery.

6.1.2 Decontamination protocols

If 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 and keep away from hazards such as power lines
- Waste water, soil or plant residues should be contained (see PLANTPLAN 2010 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

6.1.3 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

6.1.4 Plants, by-products and waste processing

- Seeds harvested from infected plants and any soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial (in a non-cropping area).
- As the pathogen can be mechanically transmitted, killed crops should be ploughed in or burnt to prevent the spread of dead, infected plant material.
- Infested paddocks should remain free of susceptible host plants until soil has been shown to be free from the pathogen. Given that both cereals and legumes are able to host the fungus to a degree (Johansson *et al.*, 2006) further work is needed to determine species or cultivars of a species that do not act as a host for the fungus.

6.1.5 Disposal issues

- Particular care must be taken to minimise the transfer of soil or plant material from the area as *V. longisporum* 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

6.2 Quarantine and movement controls

6.2.1 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

6.2.2 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, 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

6.3 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.

6.3.1 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 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.

6.3.2 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.

6.3.3 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.

6.3.4 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.

6.3.5 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 suspect 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.

6.4 Decontamination and farm clean up

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

6.4.1 Decontamination procedures

General guidelines for decontamination and clean up:

- Refer to PLANTPLAN (Plant Health Australia 2011) for further information
- Keep traffic out of affected area and minimise 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 6.1.2
- 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)

6.4.2 General safety precautions

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

6.5 Surveillance and tracing

6.5.1 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 *V. longisporum* 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

6.5.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (see Section 6.3), 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 3 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 3. Phases to be covered in a survey plan

Phase 1	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Preliminary survey of host crops in properties in buffer zone establishing points of pest detection
Phase 3	<ul style="list-style-type: none"> Surveillance of an intensive nature, to support control and containment activities around points of pest detection
Phase 4	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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-borne dispersal of the pest during these weather events
Phase 5	<ul style="list-style-type: none"> Surveillance of nurseries, gardens and public land where plants known to be hosts of pest are being grown
Phase 6	<ul style="list-style-type: none"> Agreed area freedom maintenance, post-control and containment

6.5.3 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.
- 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 *V. longisporum* to be undertaken for a minimum of 10 years after eradication has been achieved

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7.1 Websites

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

8 Appendices

8.1 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).

8.2 Appendix 2. Resources and facilities

Formal diagnostic services for plant pests in Australia are delivered through a network of facilities located in every state and territory. These services are provided by a range of agencies, including state and territory governments, the Australian Government, commercial and private diagnostic laboratories, museums, CSIRO and universities. A current listing of these facilities can be found at www.npbdn.net.au/resource-hub/directories/laboratory-directory

The national network is supported by the Subcommittee on Plant Health Diagnostic Standards (SPHDS), which was established to improve the quality and reliability of plant pest diagnostics in Australia. SPHDS also manages the production of National Diagnostic Protocols.

For more information on the diagnostic services, or to identify an appropriate facility to undertake specific pest diagnostic services, refer to www.npbdn.net.au or contact the SPHDS Executive Officer on SPHDS@daff.gov.au

8.3 Appendix 3. Market access impacts

Within the DAFF MICorP (plants) database, no countries appear to have a specific statement regarding area freedom from *V. longisporum* (January 2013). Should *V. longisporum* be detected or become established in Australia, some countries may require specific declaration. Latest information can be found within MICorP (plants) using a search for the particular pest.

MICorR (plants) contains information about the conditions to export plant and plant products. For further information on MICOR see website at <http://www.daff.gov.au/micor/plants>