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Review

Black root rot of cotton in Australia: the host, the pathogen and disease management

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Abstract. Black root rot is a seedling disease caused by the soil-borne fungal pathogen *Thielaviopsis basicola*, a species with a worldwide distribution. Diseased plants show blackening of the roots and a reduced number of lateral roots, stunted or slow growth, and delayed flowering or maturity. It was first detected in cotton in Australia in 1989, and by 2004, *T. basicola* reached all cotton-growing regions in New South Wales and Queensland and the disease was declared as an Australian pandemic. This review covers aspects of the disease that have implications in black root rot spread, severity and management, including the biology and ecology of *T. basicola*, host range and specificity, chemical and biological control of *T. basicola* in cotton cropping systems, and crop rotations and host resistance. This review is of special interest to Australian readers; however, the incorporation of ample information on the biology of the pathogen, its interactions with plants and it relation to disease management will benefit readers worldwide.

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Australian cotton

Cotton belongs to the genus *Gossypium* in the Malvaceae family (USDA 2013*b*), and of the 17 native species of *Gossypium* in Australia (Brubaker *et al*. 1999), none produces significant fibre for commercial production. The main commercial cotton grown in Australia today is derived from *Gossypium hirsutum* (upland cotton, the majority of commercial cotton in Australia) and *Gossypium barbadense* (pima cotton), which were introduced to Australia as a source of textile fibre from the Americas (Brubaker *et al*. 1999). Historically, in 1788, Governor Phillip brought seed for commercial cotton production to Australia and cotton was exported for the first time in 1831. The shortage caused by the American Civil War of 1862–65 increased the demand for cotton produced in the state of Queensland (Healy 1923); however, only with the development of irrigation in the 1960s was a stable Australian cotton industry established in northern New South Wales (NSW) and southern Queensland (CCC-CRC 2012*a*). Currently, 95% of Australia's cotton growers plant transgenic varieties (CCC-CRC 2012*a*) and Australian production is the sixth largest in the world, standing at 4.5 million 480-lb bales, with the highest yield worldwide of 4645 lb (2107 kg) per hectare in 2012 (USDA 2013*a*).

Black root rot of cotton in Australia

Black root rot is a seedling disease caused by the soil-borne fungal pathogen *Thielaviopsis basicola*, a species with a worldwide distribution (Nag Raj and Kendrick 1975; USDA 2013*b*), with the first reported case on cotton in Sacaton, Arizona, in 1922 (King and Presley 1942). Diseased cotton plants show stunted or slow growth early in the season compared with surrounding healthy plants, and delayed flowering or maturity. Belowground symptoms include blackening of the roots and reduced number of lateral roots (Allen 2001).

Although there were no records of root rot in the first 200 years of cotton growing in Australia, it was known as a serious cotton disease in North America, and good crop management was recommended (Healy 1923). In 1930, *T. basicola* was first observed in Australia, when it was isolated in Queensland from sweet pea, which was grown in the cooler season (Simmonds 1966). In 1981–82, it was reported from other plants in Australia, including tobacco, bean and pine (Warcup and Talbot 1981 and Sampson and Walker 1982, cited in Allen 1990). It was first detected in cotton in 1989 in north-western NSW (Allen 1990), and since then the pathogen has quickly spread to all cotton-growing areas of NSW, most likely by movement of pathogen spores attached to footwear, equipment or machinery. By 2004, *T. basicola* reached all cotton-growing regions in NSW and Queensland, and the disease was declared an Australian pandemic (Nehl *et al*. 2004*a*). Black root rot has had a significant impact on the Australian cotton industry, with delayed crop maturity and yield losses as high as 1.5 bales per acre (i.e. 705 bales per ha) (Jhorar 2004). The 2010–11 cotton pathology surveys (Allen *et al*. 2012*a*) showed that black root rot was found in 93% of the farms and 83% of the fields surveyed in NSW.

The origin of *T. basicola* in Australia is unclear. The disease has been recorded on a wide range of agriculturally and horticulturally important species, both native and exotic (Honess 1994) and has been reported in all states except the Northern Territory. Nevertheless, it is probably not endemic, as despite extensive screening there are no records of natural occurrence of *T. basicola* in undisturbed Australian soils

(Pattemore and Aitken 2000; Harvey *et al*. 2003). This is in contrast to its presence in uncultivated soil and plants (not always pathogenic to plants) across Europe and the USA (Yarwood 1981). It is possible that the pathogenic strains found on cotton crops were introduced to Australia via the importation of cotton-processing machinery from California (Honess 1994) or in peat (Graham and Timmer 1991). It has been suggested that the infection of native species probably occurred through horticultural practice, using peat or potting mix infected with the pathogen, since peat moss or peat-based media has been identified as a source of infection in greenhouse nurseries (Graham and Timmer 1991; O'Brien and Davis 1994). *Thielaviopsis basicola* has been recovered from greenhouse air samples (Graham and Timmer 1991) and can be spread by insect vectors (Stanghellini *et al*. 1999; El-Hamalawi 2008*a*, 2008*b*).

In addition to *T. basicola*, other cotton seedling pathogens, including *Rhizoctonia*, *Pythium*, and *Fusarium* are among the major causes of cotton seedling mortality, which reached 36% in 2011 in NSW (Allen *et al*. 2012*a*). Lesions caused by *T. basicola* may open up the roots for infection by other seedling pathogens that can cause mortality (Allen *et al*. 2012*b*); however, it is unclear whether *T. basicola* alone can cause mortality in cotton. There are some indications in the literature that, under favourable conditions, *T. basicola* can kill cotton seedlings at the later stages of their development (Hillocks 1992). It has been shown to cause vascular necrosis in the presence of the root-knot nematode, *Meloidogyne incognita*, in cotton in the USA, leading to increased mortality of cotton seedlings compared with either pathogen alone (Walker *et al*. 1998). No documentation has been found of *M. incognita* or other root nematode associated with black root rot in Australia (L. Pereg, unpubl. data). Plant parasitic nematodes of the species *Helicotylenchus dihystera* were found within roots of Australian cotton (*G. hirsutum*) without any correlation to fungal disease (Knox *et al*. 2006). However, a relationship of *T. basicola* with nematodes or other pests affecting roots cannot be ruled out. While disease surveys in Australia showed that there is no general association between black root rot incidence and seedling mortality, black root rot has been shown to cause a decrease of up to 46% in yield in experimental fields in Australia (Nehl *et al*. 2004*a*).

Distribution and prevalence

Regular disease surveys of cotton fields in NSW have shown a dramatic increase in the incidence of black root rot caused by *T. basicola* since it was first observed on cotton in Australia in 1989 (Allen 1990). Cotton disease occurrence and severity on farms, in fields within farms, and on plants within fields has been assessed in Australia in all cotton-growing regions for over a decade (Allen *et al*. 2012*a*; CCC-CRC 2012*b*). Black root rot has been surveyed yearly in Murrumbidgee, Lachlan, Macquarie, Namoi, Gwydir, Macintyre, Bourke/Walgett (NSW), Darling Downs, St George, Theodore/Moura, Emerald and from 2009–10 in Burdekin (Queensland). Surveys since the 2004–05 cotton-growing season show that incidence and disease severity are higher in NSW than in Queensland. In NSW, while all of the growing areas tested have the disease, the Namoi and Macquarie have shown the highest prevalence of black root rot consistently since 2004. Disease severity in the major cotton-growing valleys in NSW (Macintyre, Gwydir, Namoi and Macquarie) has been relatively steady between the 2004–05 season, with 66% of fields and 24% of plants surveyed showing disease, and the 2008–09 season, with 65% of fields and 32% of plants showing disease. In the 2009–10 season, 93% of farms visited and 58% of the fields surveyed in NSW were affected by black root rot, and in 2010–11 the proportions increased to 93% of farms visited and 83% of the fields surveyed in NSW. In Queensland, although the disease was reported to be present in all cotton-growing areas except Burdekin, disease prevalence was relatively low, and at least since 2004–05, black root rot has not been detected in Theodore and Emerald (CCC-CRC 2012*b*).

The lower prevalence of the disease in Queensland than in NSW is most likely due to the higher temperatures in Queensland at the start of the cotton-growing season. Similarly, in Arizona, black root rot has been more prevalent at higher elevations, where soil temperatures are cooler at planting, than at lower elevations (Mauk and Hine 1988). The disease appears to be most severe early in the growing season when soil temperature is <24°C (Rothrock 1992); high soil water content and poorly drained soils were also reported to enhance disease severity (King and Presley 1942; Rothrock 1992). As soil temperatures increase later in the season and plants resumes growth, the diseased cortical tissue sloughs off the dead cortical cells and roots elongate (King and Presley 1942; Mathre *et al*. 1966; Mauk and Hine 1988). It is noteworthy that this sloughing-off of the infected tissue leaves the root white, potentially causing marked underestimation of disease prevalence if field surveys are delayed. This was suggested to be the case with the recording of disease in Queensland in the 2010–11 season, as surveys were delayed due to severe flooding (Allen *et al*. 2012*a*). In addition to disease severity, the survival of spores of *T. basicola* also depends on soil parameters, including texture, temperature and moisture (Rothrock 1992), with lower survival of spores at higher temperature of 24−28°C than at 10−18°C. A similar trend was described in both naturally infested and inoculated soils (Papavizas and Lewis 1971; Rothrock 1992).

Movement of fungal spores with irrigation water was suggested as a possible explanation for the steady increase in the prevalence of the disease within farms and fields in Australia (Nehl *et al*. 2004*a*). An increase in disease prevalence has been observed constantly in black root rot surveys before 2004 (Nehl *et al*. 2004*a*) and from the 2004–05 to 2010–11 seasons (CCC-CRC 2012*b*). Inoculum of *T. basicola* was observed in irrigation water and in floating crop residues (Nehl *et al*. 2004*a*). Spores of *T. basicola* may be dispersed attached to soil adhering to the floating residues or possibly in the vascular tissue of the cotton residues (Nehl *et al*. 2004*a*). The latter may be possible if internal colonisation of mature plant stems occurs, as may happen occasionally (King and Presley 1942; Mauk and Hine 1988), and might release large amounts of reproduction bodies of *T. basicola* to the soil. The incidence of black root rot in the largest cotton production areas in NSW could have a high impact on the Australian economy, as Australia has been one of the world's largest cotton exporters (Dowling 2003; USDA 2013*a*).

The fast spread of the disease in Australian cotton is rooted in the biology of both the pathogen *T. basicola* and its cotton host.

Understanding the way the pathogen is interacting with the plant, and factors that enhance or suppress the pathogen growth and disease severity in cotton, could lead to improved integrated management of black root rot.

The biology of *T. basicola* and its interaction with plants

Thielaviopsis basicola is a soil-borne, filamentous, hemibiotrophic fungus (Mims *et al*. 2000). Fungal hemibiotrophs start their infection cycle with a biotrophic phase and then move to a necrotrophic phase. Although *T. basicola* is generally considered an obligate parasite (Hood and Shew 1997), it can also associate with hosts in a non-pathogenic manner (Yarwood 1974) and is capable of limited saprophytic utilisation of soil organic matter (Gayed 1972; Chittaranjan and Punja 1994).

The life cycle of *T. basicola* has been described for tobacco (Hood and Shew 1997), pansy (Mims *et al*. 2000) and cotton (Mauk and Hine 1988). Based on these descriptions, the cycle can be divided into six major steps: (*i*) germination of spores; (*ii*) growth of the germ tube towards roots; (*iii*) attachment to root surface—the first contact by the pathogen and initial host–pathogen recognition; (*iv*) differentiation of the pathogen into infection structures and penetration into the host cells; (*v*) establishment of a biotrophic phase; and (*vi*) conversion to necrotrophy (root rotting) and the production of new spores. Changes in both organisms during each step of the cycle indicate constant communication between the host and the pathogen.

Communication between cotton host (*G. hirsutum*) and *T. basicola* early in the infection cycle was detected using two-dimensional gel electrophoresis separation of cotton root proteomes (Coumans *et al*. 2009, 2010). It was shown that (*1*) new proteins are expressed in the cotton root as early as 1 day after infection with *T. basicola*, and (*2*) *T. basicola* is capable of adapting its proteome to germinate and grow according to nutrients available in its environment (Coumans *et al*. 2009, 2010). Moreover, another study by the same group on the interaction of *T. basicola* with its hosts showed that germ tubes and hyphae of *T. basicola* grow specifically in the direction of the host when challenged with germinating cottonseeds (Pereg 2011).

Thielaviopsis basicola produces two types of spores, endoconidia (also known as phialospores) and chlamydospores (also known as aleuriospores). Figure 1*a* presents the two types of spores produced by *T. basicola*. In culture, endoconidia are produced within 24 h and chlamydospores within 3 days (Shew and Meyer 1992), with some variations depending on the isolate and culture conditions. The presence of the two types of spores and the shape of the chlamydospores are good morphological tools for the identification of the species. Since the endoconidia have all the essential features found in the *Chalara* complex, Nag Raj and Kendrick (1975) have placed *T. basicola* in the genus *Chalara*, with the species name *Chalara elegans*. However, DNA sequencing techniques have shown that it belongs to the species *T. basicola*, and *C. elegans* is now considered a synonym of *T. basicola* (Paulin-Mahady *et al*. 2002).

The endoconidia are produced from phialides, and liberated single endoconidia are hyaline, cylindrical with rounded ends and variable in size (Delvecchio *et al*. 1969) and can only survive in the soil for a few months, with <1% survival in the soil after 15 months (Schippers 1970). The chlamydospores are produced at the tip of hyphae in chains composed of thick-walled melanised (dark) compartments surrounded by a distinct outer wall (Delvecchio *et al*. 1969). Their thick walls and the presence of melanin protect them from adverse conditions such as extreme temperatures, low moisture, UV radiation and microbial lysis, allowing them to survive in the soil for years as resting spores (Tsao and Bricker 1966). The persistence of the stress-resilient chlamydospores in the soil makes black root rot so hard to eradicate once it reaches a field. Control measures to enhance sanitation, such as ‘come

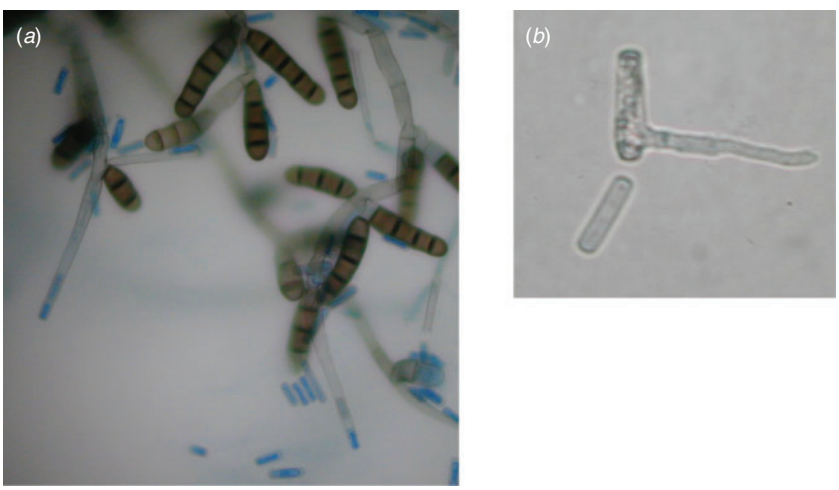

Fig. 1. Spores produced by *Thielaviopsis basicola*. (*a*) Chlamydospores (large, thick-walled, brown chains) and endoconidia (small, cylindrical thin-walled and stained blue with Cotton Blue) produced by *T. basicola*, and (*b*) endoconidial germination in culture. Each single chlamydospore in the chain measures ~10–16 µm in length and 5–8 µm in width. Endoconidia appear cylindrical, truncated at each end, and measure 8–20 µm in length and 4–6 µm in width.

clean go clean' (Maas 2011; Allen *et al*. 2012*b*), have been recommended; however, these methods have little effect once the pathogen reaches a farm, in particular where irrigation is employed as discussed above. There are limited management options currently available for reducing black root rot (Table 1).

Germination of fungal spores is thought to occur in response to signalling, either physical (thigmotropic) or chemical (Prell and Day 2001). Figure 1*b* presents germinated spores of *T. basicola* in culture. Endoconidia and chlamydospores of *T. basicola* were shown not to germinate in fallow soil (Papavizas and Adams 1969) but germinated in the presence of root extracts, some sugars or specific stimulatory substances such as natural lecithins or their constituents, unsaturated fatty acids or unsaturated triglycerides (Mathre and Ravenscroft 1966; Papavizas and Adams 1969). Lindeman and Tousson (1968) showed that germination of *T. basicola* only occurred in the immediate vicinity of the host (*G. hirsutum*). In addition, spores of a cotton isolate of *T. basicola* germinated and reproduced in response to exudates from several hosts (cotton and some legume hosts), including wheat, which had been considered a non-host, as it did not show disease symptoms (Rothrock and Nehl 2000). A decade later, laboratory experiments have shown that wheat can host *T. basicola* (Pereg 2011) and this will be discussed below.

Root hairs are the primary penetration sites for *T. basicola*, but penetration has also been reported through other root epidermal cells (Jones 1991; Nan *et al*. 1992) and through wounds (Baard and Laubscher 1985; Punja *et al*. 1992). Histological studies revealed that, in cotton seedlings, *T. basicola* invades the root cortex (Mathre *et al*. 1966). Root invasion occurs during the first 2–8 weeks of the cotton growth (Hillocks 1992), causing disease symptoms in crop, whereas older roots appear to be more resistant to infection.

Histological studies of the infection process of susceptible and resistant cultivars of tobacco by *T. basicola* indicated a dynamic interaction in the establishment of the parasitic relationship (Hood and Shew 1997). Spore germination, germ tube growth and penetration of root tissue were similar in the two cultivars, with penetration of root hairs and epidermal cells observed, with epidermal cells the most commonly observed site of infection (Hood and Shew 1996). However, hyphae advancement and lesion development were limited in the resistant cultivar, whose root system outgrew the effects of the initial inoculation (Hood and Shew 1996). In cotton, *in vitro* studies at 24°C have shown that endoconidia germinated within 6 h of inoculation onto *G. barbadense* seedling roots and penetration of host tissue occurred within 12 h after inoculation; chlamydospores germinated after 24 h of incubation and host tissue was penetrated within 36 h (Mauk and Hine 1988). Tissue colonisation occurred immediately after penetration, and 10 days after inoculation and incubation, seedlings were stunted, roots were decayed, and the height of the plants was significantly reduced compared with controls (Mauk and Hine 1988). Production of chlamydospores is associated with the necrotrophic phase, and they are produced throughout the root cortex and on the surface of the roots and adjacent soil. The vascular tissue of roots is usually not invaded (Walker *et al*. 1998), allowing for the survival of the plant host (Hood and Shew 1997; Mims *et al*. 2000).

Thielaviopsis basicola demonstrates diverse phenotypes when grown in culture (Fig. 2). Colony pigmentation of *T. basicola* has been described as grey, olive, dark blackish brown or black (Stover 1950; Ellis 1971). Other than brown and grey types, a white (albino) phenotype and 'sectoring' of older colonies were observed, where pigmented types developed albino sectors with each variant capable of giving rise to the other during subculturing (Punja and Sun 1999). Moreover, differences in colony appearance, colour, growth rate, production of spores, length of chlamydospore chains and virulence have been observed within axenic cultures grown from single chlamydospore (Huang and Patrick 1971). The number and size of individual spores in the chlamydospore chains vary among isolates of *T. basicola* (Punja and Sun 1999). Moreover, differences have also been observed among adjacent spores in the same chlamydospore chain. These morphological variants appear to arise frequently during growth in culture of some wild-type isolates and may show variations in pathogenicity (Huang and Patrick 1971). Isolates within a morphological group were not unique to any given geographical region or host of origin (Punja and Sun 1999). Variation in colony morphology, colour and size has also been observed when growing *T. basicola* in the presence of different plant root extracts (Coumans *et al*. 2010). Therefore, substantial care is required when attempting to classify *T. basicola* according to its appearance in culture.

Sexual reproduction has not been demonstrated in *T. basicola* (Paulin-Mahady *et al*. 2002) and it is not known whether isolates may include more than one mating type (Geldenhuis *et al*. 2006). Since a teleomorph of the barley pathogen *Septoria passerinii* was found ~125 years after the description of the anamorph (Ware *et al*. 2007), it is possible that a cryptic sexual cycle of *T. basicola* may be found, and if it exists, it may explain the high variation observed within cultures of the same isolate and the variations among isolates. The genetic basis for the morphological differences is unknown; however, they may have evolved as a strategy for improving the long-term survival of *T. basicola* in adverse conditions or new environments.

Australian isolates from two different cotton-growing regions clustered into distinct regional groups when analysed for genetic variability using random amplification of polymorphic DNA (RAPD) (Pattemore and Aitken 2000). Lettuce and peat isolates were also found to cluster together and were distinct from other host groups, indicating that peat was probably the source of *T. basicola* found in lettuce soils. The isolate from tobacco soil clustered separately from isolates from other hosts (Pattemore and Aitken 2000). Isolates from similar geographic regions or hosts formed distinct groups when analysed for genetic variability using hierarchical clustering analysis of RAPD-PCR results (Punja and Sun 1999). The fact that different isolates of *T. basicola* from the same host share greater genetic similarity and morphology than those from different hosts indicates that host selection was likely to be determined by genotype (Punja and Sun 1999).

It has been suggested that the high intra-specific variation of strains of *T. basicola* may be due to a high mutation rate and/or the presence of transposable genetic elements (Punja and Sun 1999) and that *T. basicola* may contain double-stranded RNA

Table 1. Management strategies for controlling black root rot: potentials and limitations

The information in this table is summarised from the review text. All references are given throughout the text, including: Allen *et al*. 2012*b*; Maas 2011; Matthiessen and Kirkegaard 2006; Nehl *et al*. 2004*a*, Nehl *et al*. 2004*b*; Jhorar 2004; Harrison and Shew 2001; Wheeler *et al*. 1999; Brubaker *et al*. 1999; Candole and Rothrock 1998; Zaki *et al*. 1998; Kaufman *et al*. 1988; Wheeler *et al*. 1997; Arthur 1996; Butler *et al*. 1996; Rothrock 1992; King and Presley 1942

Management tools	Mechanisms/potential	Limitations
Monitoring for disease and ensuring awareness	Awareness of the presence of the pathogen would lead to informed crop production practices and disease management. It requires the identification of plants that show signs of poor vigour or unusual symptoms in the field as well as examination of seedling roots for the typical blackening and presence of *T. basicola* typical spores (Fig. 1)	Plants that are badly affected early in the season may not continue to show symptoms later in the season as the infected tissue may slough off when growth resumes in warmer weather. Nevertheless, the spores released into the soil increase the soil reserves of the pathogen. In following seasons, one may no longer observe patches of stunted growth as the spores may have spread and the entire field might be infected
Planting varieties that can catch up later in the season	Black root rot on its own does not kill plants. As the season progresses and the temperatures increase, the plant may overcome the seedling disease and growth may catch up given appropriate varieties planted	Selection of varieties for planting is often determined by a range of factors, such as cost, presence of pests, weeds or other diseases (fusarium or verticillium wilt) and grower's preference
Applying acibenzolar-S-methyl (Bion™) seed treatment	Induces host systemic resistance against black root rot and other pathogens	Tests gave mixed results in Australia, with disease suppression of up to 33% observed. It cannot control black root rot on its own but is recommended for use with other treatments in warmer seasons when disease pressure is moderate
Irrigation scheduling: pre-irrigation and/or planting into moisture	Lower water potential seems to reduce disease so water logging should be avoided at all times. Applying water before planting provides better conditions for seedling emergence than watering after planting. Monitoring signs of water stress, especially if root system has been weakened by disease early in the season, would allow appropriate irrigation planning	Due to unpredictability of weather conditions it is not always possible to avoid irrigation or watering after planting
Delaying planting to avoid cool periods; maintaining appropriate soil nutrition levels	The disease appears to be most severe early in the growing season when soil temperature is <24°C. Delaying planting time would avoid cool conditions that favour the disease early in the season. Increased temperatures and balanced nutrition promote plant growth and assist plants to overcome the disease	Grower routine and preferences in planting times and in application of fertilisers. Good planning requires performing nutritional analysis of the soil
Avoiding bare fallow for more than one season	Bare fallow does not decrease the *T. basicola* spore load in the soil. Bare fallows for 16–18 months did not seem to reduce mycorrhizal development in cotton in northern regions of Australia. Although periods of very long bare fallows with wetting and drying cycles with no rotation crop or weed growth may result in a deficiency of arbuscular mycorrhiza (AM deficiency), such conditions are unlikely to occur in Australia. It is worth noting that since cotton is highly dependent on AM, AM deficiency might compromise the plant health, making it more prone to disease. A mycorrhizal-rotation crop may restore sufficient AM for cotton. A review dedicated to the survival and importance of AM in cotton production systems in Australia is required to clarify this important issue for cotton growers	
Rotation with non-hosts for up to 3 years	*Thielaviopsis basicola* is a hemibiotrophic fungus, requiring a live host to complete its life cycle and produce new spores. Growing non-hosts may reduce the fungal load in the soil since spores germinating in response to root exudates may not find a host for completion of the reproduction cycle	Field experiments show mixed results with cereals, which were considered non-hosts. Some host plants may be mistaken for non-hosts since they do not show symptoms of disease even when the pathogen infects their roots and produces spores (non-susceptible hosts). Under field conditions, if used as rotation crops, they might enhance/maintain the pathogen spore load in the soil. Non-hosts of *T. basicola* might be hosts for other pathogens that cause disease in cotton
Avoiding legumes; controlling alternative hosts and volunteer cotton	Legume (faba bean, soybean, cowpea, field pea, chickpea, mungbean, lablab, lucerne and others) and some weeds (thornapple, castor oil) are hosts to *T. basicola* and can increase the fungal load of the soil. Controlling alternative hosts would prevent build-up of inoculum and carryover of disease from one season to the next	

(*Continued next page*)

Table 1. (*continued*)

Management tools	Mechanisms/potential	Limitations
Effective biofumigation with vetch or mustard	Vetch fixes substantial quantities of nitrogen and its degradation produces ammonium. High levels of ammonium are suppressive to *T. basicola*, probably since plants exposed to high levels of ammonium produce substances that are toxic to *T. basicola* (e.g. putrescine). Other reasons could include an increase of the soil pH by the ammonium released	Biofumigation crops may reduce the incidence of *T. basicola* infection, but may be hosts for other cotton pathogens. Vetch residues can increase the activity of fusarium wilt in the following cotton crop. The success of biofumigation depends on the growth of the biofumigant crop and timely incorporation. Despite its demonstrated potential, the capacity of hairy vetch to reverse severe infestation of *T. basicola* has not been proven in fields where cotton is cropped regularly in Australia
Practicing good farm hygiene; managing crop residues; minimising tail-water	Cleaning soil and crop debris from vehicles, machinery and footwear and applying an appropriate disinfectant before coming onto, or when leaving, a farm would reduce spore spreading between fields and farms. Correctly disposing of crop byproducts, residues and trash would minimise carryover of pathogens into subsequent crop and movement of crop residues in tail-water recirculation systems. Retaining tail-water and runoff water on farm and keeping it out of river systems would reduce spread	The thick walled spores (chlamydospores) produced by the pathogen can survive in the soil for years and start the disease cycle once a host is available. Therefore, control measures to enhance sanitation, such as 'come clean, go clean', are essential for disease management; however, these methods have little effect once the pathogen reached a field, in particular where irrigation is employed
Summer flooding if possible	Flooding for 30–60 days before planting seems to reduce disease in the next crop (can decrease the severity of black root rot by up to 98%, especially when applied before planting)	In Australia, summer flooding is constrained not only by high costs but also by terrain and the availability of water, and due to the risk of disease spread through runoff (from flood and irrigation)
Optimisation of seed bed conditions	Planting into well-prepared, firm, high beds would optimise stand establishment and seedling vigour. Any damage to the root would enhance disease, so avoiding damage by appropriate placement of fertiliser and herbicides in the bed and having good drainage, not allowing water to back-up and inundate plants, would contribute to disease control	
Fungicide treatment	In the USA, fungicide seed treatments (e.g. myclobutanil and triadimenol) were found to be effective in years with cool and wet early seasons. Cotton yield increased when seed was treated with a mix of triadimenol, captan and metalaxyl, and cotton stands increased significantly using a commercial mixture of the fungicides metalaxyl, triadimenol, and thiram	To date, fumigation has not been a practical control measure for Australian cotton farms since fumigants do not penetrate and disperse well in the clay soils common to Australian cotton farms, and in-furrow application of several fungicides has been shown to be ineffective. Furthermore, some fungicides have been shown to have a phytotoxic effect on cotton, delaying emergence and slowing plant growth
Breeding for resistance	There are resistant diploid cotton cultivars that could be used in crossbreeding	Being distant diploid relatives of the commercial tetraploid cottons makes crossbreeding for resistance difficult. There is always the chance that new cultivars resistant to one disease might show increased susceptibility to other diseases, so care must be practiced before releasing new cultivars
Transgenic plant with antifungal genes	There are some promising defence genes being tested for disease resistance but further research is needed before an optimal transgenic plant resistant to black root rot can be produced	There is no transgenic variety resistant to black root rot available. When dealing with a seedling disease, it may be a disadvantage to introduce a defence gene that will be expressed throughout the life of the plant and increase the metabolic load beyond the seedling stage during boll production
Biocontrol	Antagonistic and plant growth promoting microbes may inhibit the growth of the pathogen or suppress disease through different mechanisms (e.g. antibiosis, competition for infection sites or resources, improving plant nutrition and inducing plant systemic resistance)	Biocontrol products for managing black root rot are not currently available in Australia. Any new and imported products will have to be tested for their efficiency under local conditions

(dsRNA) of viral origin, although some strains may carry two or more distinct, serologically unrelated viruses (Subramanian 1983). The presence of the virus seems to have no profound effect on the growth or morphology of the host, or on pathogenicity of the fungus (Subramanian 1983), although altered fungal physiology and virulence have been reported (Bottacin *et al*. 1994; Punja 1995). There is no information on the presence of dsRNA viruses in *T. basicola* in Australia. There is a gap in the information on the genotypes of *T. basicola* existing in Australia; analysis of isolates from fields across cotton-growing areas in NSW and southern Queensland (L. Pereg, S. Cooper and K. Kirkby, unpubl. data) will shed light on the diversity of this

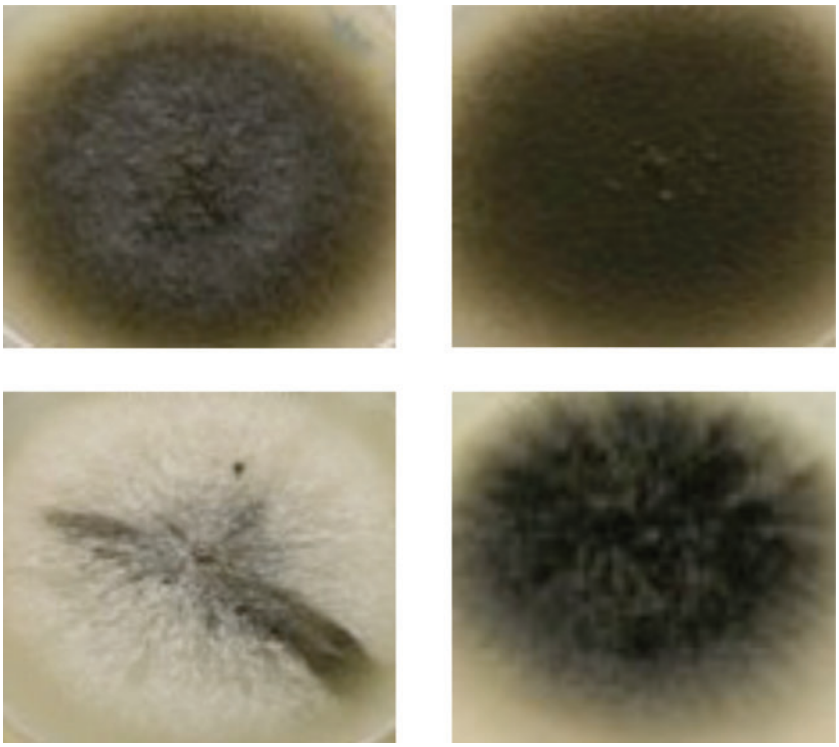

Fig. 2. Four isolates of *Thielaviopsis basicola* displaying various morphologies in culture. Top left, cotton isolate; top right, carrot isolate; bottom left, lettuce isolate showing an albino variant; bottom right, lupin isolate.

pathogen in Australia and the virulence of distinct isolates towards cultivated cotton.

In a recent study using genomics (internal transcribed spacer sequence analysis, ITS) and proteomics (total protein mapping) tools, various isolates of *T. basicola* were grouped according to host of origin, namely cotton, carrot or lettuce, irrespective of geographical origin (Coumans *et al*. 2011). Evidence is accumulating to suggest that isolates of *T. basicola* show some degree of host specificity or preference (Meyer and Shew 1991; O'Brien and Davis 1994; Coumans *et al*. 2011; Pereg 2011), which may lead to differentiation of isolates of *T. basicola* into intra-specific groups.

Host range and host specificity and their implications for disease management

Other than persistence of the resilient spores of *T. basicola* in soil, one of the reasons that black root rot is hard to control is the wide host range of the pathogen; *T. basicola* has a host range of >230 species (Hood and Shew 1997; Farr and Rossman 2013) and is commonly found on plants of the Fabaceae, Malvaceae, Solanaceae and Cucurbitaceae families (Otani 1962; Yarwood 1974, 1981; Shew and Meyer 1992; Subramanian 1968). Of particular importance is the fact that it causes disease on several crop plants, e.g. cotton (Mathre *et al*. 1966), tobacco, several grasses (Gayed 1972), groundnut, bean (Tabachnik *et al*. 1979), chicory (Prinsloo *et al*. 1991), carrots (Punja *et al*. 1992), citrus (Graham and Timmer 1991), tomato (Koike and Henderson 1998) and pineapple (Wilson Wijeratnam *et al*. 2005). Ornamental plant species affected include pansies (*Viola carnula*), sweet pea (*Lathyrus odoratus*) and *Nemesia* (O'Brien and Davis 1994).

Cotton and carrot isolates of *T. basicola* were significantly more virulent towards cotton than were isolates from lettuce (Coumans *et al.* 2011), and these differences in virulence were associated with proteomic differences between cotton, carrot and lettuce isolates. Two lettuce isolates tested on 13 hosts of *T. basicola* were found to have clear differences in the susceptibility of 11 lettuce cultivars tested (O'Brien and Davis 1994). The same study showed that disease on bean plants was severe, and on watermelon, cucumber and rock-melon moderate or low, whereas other plants such as eggplant and capsicum showed complete resistance to black root rot. In this trial, the cotton cultivar *Gossypium hirsutum* cv. Siokra-1-4 showed no disease (O'Brien and Davis 1994). Seven different isolates of *T. basicola* caused highly variable disease severity on a susceptible tobacco cultivar (Meyer and Shew 1991). In other studies, isolates of *T. basicola* highly pathogenic on poinsettia have been found to be moderately pathogenic on beans and non-pathogenic on tobacco, whereas tobacco isolates were found to be non-pathogenic on beans and poinsettia (Keller and Shanks 1955; Lloyd and Lockwood 1963). Although isolates of *T. basicola* from Australian cotton are not limited to causing black root rot on cotton and have also been found to be highly pathogenic towards lupin, pansy and soybean, these isolates are non-pathogenic towards lettuce (Mondal *et al*.

2004; Pereg 2011), thus displaying host specificity that could possibly be exploited in the search for fungal pathogenicity factors and mechanisms of host resistance.

Thielaviopsis basicola exhibits three modes of interactions with plants (Pereg 2011); isolates may (*1*) infect the roots and cause disease; (*2*) infect the roots but not cause disease; or (*3*) not infect the roots. Pursuant to this organisation, it was suggested that plants be placed into three categories: susceptible hosts, non-susceptible hosts, and non-hosts to *T. basicola*. Non-susceptible hosts are those in which chlamydospores of *T. basicola* were detected on healthy-looking roots of the plants. It is unclear whether such infected hosts would remain unaffected by the disease under any given conditions. Nevertheless, it was found that wheat is a non-susceptible host to several isolates of *T. basicola*, including cotton isolates when tested in soil under controlled condition (Pereg 2011).

Rotation with non-host crops, especially monocots, has been shown to reduce the density of *T. basicola* in the soil and the incidence of black root rot (Holtz and Weinhold 1994). That study found larger populations of *T. basicola* in soil in California planted for ≥3 years to cotton than in soil rotated to other crops including barley, wheat or safflower (Holtz and Weinhold 1994). Hairy vetch (*Vicia villosa*) can be planted during winter as a cover crop to possibly improve soil properties (Rogers and Giddens 1957); seed cotton yield increased by up to 162 kg ha^{-1} year^{-1} in a cotton production system with legume as a cover crop (Scott *et al*. 1990). Rotation with other host plants, such as planting soybean in cotton farming systems, may contribute to the cumulative increase of inoculum with time (Mondal *et al*. 2004). Rotation with a non-host for up to 3 years is one of the current recommendations for black root rot management in cotton (Allen *et al*. 2012*b*) (Table 1). It should be noted that, previously, wheat was not considered a host, and therefore was often planted in rotation with cotton in *T. basicola*-infested fields (Nehl *et al*. 2004*a*; Coumans *et al*. 2010). The finding that wheat is a non-susceptible host (Pereg 2011) can explain the observations that black root rot severity on cotton was similar in rotation and without rotation with wheat in long-term experiments (Nehl *et al*. 2004*a*). It can also explain why the proteome response of a cotton isolate of *T. basicola* to wheat was closer to its response to the cotton host than to vetch (non-host) (Coumans *et al*. 2010). This highlights the importance of reliable techniques to analyse the interactions between the plant and the fungal isolates (Pereg 2011).

Certain plants have been identified that were more attractive to various isolates of *T. basicola* than to others, irrespective of the virulence of these isolates on these plants (Pereg 2011). This may represent a component of resistance that could be manipulated. Cotton seedlings were found to be exceptionally attractive to isolates of *T. basicola* from different origins (carrot, lettuce, lupin, cotton), including isolates that did not cause disease symptoms on cotton (Pereg 2011). A study using protein mapping and ITS analysis demonstrated that, in Australia, strains of *T. basicola* originated from descendants of a single strain or groups of closely related strains associated with specific hosts (Coumans *et al*. 2011). Accumulated evidence from these and other studies of *T. basicola*–host adaptation (Punja and Sun 1999) could explain the difficulties in controlling the disease. The question arises whether different cultivars of commercial cotton differed in their ability to stimulate hyphal growth and in their resistance to infection by various isolates of *T. basicola*, with potential use in disease management.

Current disease management options recommended for black root rot in Australia are limited to delaying the sowing time to avoid cool conditions that favour the disease early in the season, crop rotation with non-hosts, biofumigation with a green manure crop of vetch, summer flooding, seed treatment with chemicals that induce systemic resistance (acibenzolar-S-methyl) and other soil preparation techniques (Allen *et al*. 2012*b*). The development of an integrated disease management program that takes into account pathogen biology and its interactions with cotton under diverse conditions is essential for controlling infection by *T. basicola* and its persistence in the soil.

Chemical control of *T. basicola* in cotton cropping systems

Once introduced into the soil, the spores of *T. basicola* are very persistent under diverse environmental conditions. The thick-walled spores (chlamydospores) produced by the pathogen can survive in the soil for years and start the disease cycle once a host is available. The spores have been easily spread in flood and irrigation water and on cotton residues attached to machinery, equipment and footwear. Farm hygiene practices such as 'come clean go clean' (clean footwear and machinery with anti-fungal substances to reduce spore spreading) is always recommended to avoid further spreading. Some of the measures include: cleaning soil and crop debris from vehicles, machinery and footwear and applying an appropriate disinfectant before coming onto, or when leaving, a farm; correctly disposing of crop by-products, residues and trash; retaining tail-water and runoff water on-farm and keeping it out of river systems; and for the farmer to maintain communication with the relevant industry and neighbours about contamination issues (Nehl *et al*. 2004*a*; Allen *et al*. 2012*b*). However, once the spores have reached a farm, it is very difficult to eradicate them.

Soil fumigation with fungicides could possibly be used to control black root rot. The fungicides do not eradicate the fungal pathogen, but can reduce and suppress *T. basicola* present in the soil (Matthiessen and Kirkegaard 2006). In the USA, fungicide seed treatments were found to be effective in years with cool and wet early seasons. The systemic, sterol-inhibiting fungicides myclobutanil and triadimenol showed some efficacy in controlling *T. basicola* when used as cottonseed treatments (Kaufman *et al*. 1988; Arthur 1996; Butler *et al*. 1996). Other mixtures of fungicides were found to be efficient in controlling black root rot and seedling disease complexes caused by several pathogens, mainly *T. basicola* (Arthur 1996; Wheeler *et al*. 1999). Cotton yield increased when seed was treated with a mix of triadimenol, captan and metalaxyl (Wheeler *et al*. 1997), and cotton stands increased significantly using a commercial mixture of the fungicides metalaxyl, triadimenol and thiram (Zaki *et al*. 1998). To date, fumigation has not been a practical control measure for Australian cotton farms, since fumigants do not penetrate and disperse well in the clay soils common to Australian cotton farms, and in-furrow application of several fungicides has been shown to be

ineffective (Jhorar 2004; Matthiessen and Kirkegaard 2006). Furthermore, some fungicides have been shown to have a phytotoxic effect on cotton, delaying emergence and slowing plant growth (Jhorar 2004). Pre-treating seeds with fungicides such as triazole or host resistance-inducing chemicals such as acibenzolar-S-methyl (benzothiadiazole) can reduce the incidence of black root rot and improve seedling survival while avoiding the complications associated with soil treatment (Minton *et al*. 1982; Toksoz *et al*. 2009). It has shown some promise in pot trials as a method of reducing the severity of black root rot caused by *T. basicola* when used as a seed treatment immediately before sowing (Mondal *et al*. 2000). Large-scale field evaluations in cotton fields in Australia demonstrated that, while acibenzolar-S methyl as a seed treatment does not provide complete control by itself, it can induce an increased level of resistance to *T. basicola* in cotton under moderate disease pressure but it is less effective under high disease pressure (Allen 2007). It can thus be considered a valuable component of an integrated disease management strategy, especially when planting in warmer soil temperatures.

Host resistance and its potential in disease management

Although efforts have been made toward developing cotton cultivars resistant to fungal diseases, no such cultivar has progressed to the stage of commercial use. An Australian study showed that seven commercial varieties of cotton (*G. barbadense* and six cultivars of *G. hirsutum*) were all susceptible to infection by an isolate of *T. basicola* from cotton-growing soils, with no significant difference in the extent of disease apparent between the cultivars (Honess 1994). By contrast, some native Australian diploid *Gossypium* species show various levels of resistance to black root rot (Nehl *et al*. 1998), and the diploid *G. arboreum* (PI 1415) also shows high resistance to *T. basicola* (Wheeler *et al*. 1999). Partial and high resistances to *T. basicola* have been identified in *G. arboreum* and *G. herbaceum* variants, respectively (Wheeler and Gannaway 2007), and crossbreeding, followed by genetic analysis, was done in an attempt to identify quantitative trait loci of these two varieties (Niu *et al*. 2008). However, being distant diploid relatives of the commercial tetraploid cottons, crossbreeding for resistance poses some difficulties (Brubaker *et al*. 1999).

The development of transgenic plants with general antifungal genes has also been a priority for cotton industries. Magainin, an antimicrobial peptide isolated from the African clawed frog (*Xenopus laevis*), has broad-spectrum, antimicrobial activity (Kristyanne *et al*. 1997). The isoform magainin 2 has shown effects on the ultrastructure of some plant pathogens, completely inhibiting growth of *T. basicola* in culture (Kristyanne *et al*. 1997). Tobacco and banana plants transformed with a synthetic analogue of magainin, MSI-99, have been shown to have enhanced resistance to fungal pathogens (Chakrabarti *et al*. 2003), making it a strong candidate for engineering into cotton and other susceptible plant species to enhance fungal resistance. A cotton cultivar was transformed with a gene from *Trichoderma virens* (Gv29-8), encoding an endochitinase, and selected transgenic lines demonstrated significant levels of resistance against fungal pathogens, including *T. basicola* (Howell 2003).

Until recently, sources of resistance for the purposes of crossbreeding were lacking in Australia (Wang and Davis 1997; Allen 2001). More recently, there have been plans to test a genetically modified cotton line that contained a plant defensin gene, *nad1*, derived from the ornamental tobacco, *Nicotiana alata* (OGTR 2006). This gene encodes a defensin protein, NaD1, which inhibits the growth of fungi, including *T. basicola* (OGTR 2006). However, it has been reported that continuous expression of some defensins may have toxic effects, such as abnormal morphology, reduced fertility and reduced cell growth. The presence of mature NaD1 causes abnormal growth of transgenic cotton, resulting in distorted leaves and short internodes. Using a modified defensin, where NaD1 is combined with a C-terminal propeptide domain (CTPP), eliminated the toxic effects in the host plant (Anderson *et al*. 2009, cited in Stotz *et al*. 2009). Another defensin, Rs-AFP2, originating from *Raphanus sativus* (radish) has been shown to suppress several fungal pathogens *in vitro*, including strains of *Fusarium oxysporum* and *Verticillium dahliae* (Vilas Alves *et al*. 1994). A crude extract from *R. sativus* seeds has also been shown to suppress *T. basicola*. Rs-AFP2 reduces growth of some fungi by interacting with glucosylceramide in their cell membrane, increasing membrane permeability (Thevissen *et al*. 1999, 2003). Although a variety of transgenic plants containing the gene encoding Rs-AFP2 have been produced, including cotton, this did not lead to the release of a commercial product, possibly due to the toxic nature of the protein. To overcome the adverse effects that constitutive expression of defensins inflicts on transgenic plants, a line of cotton was developed expressing the *Arabidopsis* NPR1 protein (*AtNPR1*), which was shown to confer resistance against several pathogens, including *T. basicola* (Kumar *et al*. 2013). The roots of *AtNPR1*-overexpressing transgenic plants exhibited stronger and faster induction of several defence genes, particularly PR1, thaumatin, glucanase, LOX1, and chitinase, thus inducing the plant's own defence system. These transgenic plants also performed better than the wild type plant when they were challenged with *T. basicola*, showing reduced disease symptoms, significantly higher shoot and root mass, longer shoot length, and greater boll-set (Kumar *et al*. 2013). These results are promising; however, for practical applications, further studies need to be done to test NPR1-based defence technology in a more controlled manner in order to estimate the metabolic costs to the transgenic plants.

There are several points to consider in the development of transgenic plants resistant to black root rot. Once a disease-suppressive gene is successfully expressed in transgenic plants, the gene product can potentially provide protection throughout the life of the plant. Use of the *cry* genes, originally isolated from *Bacillus thuringiensis*, in cotton to protect the plant from bollworm (*Helicoverpa* sp.) is an example of this type of approach (Perlak *et al*. 2001; Pray *et al*. 2002; Pyke 2007). However, black root rot is a seedling disease, affecting the plant mainly in the first few weeks post-planting, and disease levels decline later in the growing season when temperatures increase (Rothrock 1992). Therefore, ongoing production of the anti-microbial or defence proteins is not required for the entire life span of the plant and may put an unnecessary metabolic burden on the growing plant, slowing growth and reducing yield. If a transgenic solution is sought for

seedling diseases, a system in which the introduced gene expression is switched off after several weeks should be considered. Also, taking into account the wide range of isolates of *T. basicola* that affect commercial cotton, breeding and genetic manipulation should be directed towards multiple-resistance to a mixture of strains of *T. basicola*.

Fungal ecology and its implications for disease management

Thielaviopsis basicola has a worldwide distribution and is probably a natural soil inhabitant, being found in virgin soil far removed from crops (Stover 1950; Yarwood 1981).

The severity of black root rot is primarily dependent on the susceptibility of the host plant, the strain of *T. basicola* and the inoculum concentration at the time of infection. There appears to be a positive correlation between disease severity and inoculum density (Tabachnik *et al*. 1979; Holtz and Weinhold 1994); however, disease incidence approaches maximum as the population of *T. basicola* increases above 100 cfu g^{-1} soil (Holtz and Weinhold 1994; Nehl *et al*. 2004*a*). Unfortunately, it appears that estimation of the populations of *T. basicola* alone is not an accurate tool for predicting black root rot incidence in Australian cotton-growing systems tested (Nehl *et al*. 2004*a*).

There are several other abiotic and biotic factors that have the potential to either suppress or promote *T. basicola* or black root rot and that could be manipulated in attempting to control the disease. Abiotic factors can influence the severity of black root rot caused by *T. basicola*. The optimal pH range for growth of *T. basicola* in culture is 4.0–6.5 (Punja 1993). Soil pH affects the solubility of ions in the soil, increasing it if acid, thus indirectly affecting the distribution and activity of soil microorganisms (Kaufmann and Williams 1964). Soils with pH ≥5.6 and alkaline clay soils are considered conducive, increasing black root rot severity. Soils with pH <5.2 suppress black root rot, decreasing its severity (Bateman 1962; Hillocks 1992; Meyer *et al*. 1994; Harrison and Shew 2001). Soil type may influence disease prevalence, with weathered ground moraine being suppressive and weathered molasse conducive to black root rot of burley tobacco in Switzerland (Stutz *et al*. 1989). A large proportion of cotton in Australia is produced in heavy texture, alkaline clay soil (McKenzie 1994 as reported in Nehl *et al*. 2004*a*), and black root rot in cotton is more severe in medium clay soils than in very heavy clays or lighter clays, although its severity has not necessarily correlated with water-holding capacity (Nehl *et al*. 2000).

Black root rot of cotton has been reported to be less severe in well-drained soils (King and Presley 1942). However, survival of *T. basicola* in soils with water-holding capacities of 45% is lower than in soils with ≤15% (Papavizas and Lewis 1971). Flooding as a control measure in cotton fields has been demonstrated to decrease the severity of black root rot by up to 98%, especially when applied before planting (Jhorar 2004). In Australia, summer flooding is recommended for disease management (Allen *et al*. 2012*b*) but is constrained not only by high costs but also by terrain and the availability of water, and due to the risk of disease spread through runoff.

The levels of exchangeable calcium, aluminium, nitrogen and a variety of other ions in soils can affect the severity of black root rot. Both alkaline and acidic soils containing high levels of calcium were reported to promote black root rot (Meyer and Shew 1991; Oyarzun *et al*. 1998). Acidic soils that suppress the disease generally contain high levels of aluminium, phosphates or nitrogen, reported to inhibit spore germination and hyphal growth (Meyer *et al*. 1994; Delgado *et al*. 2006). High levels of aluminium at pH ≤5.0 also inhibit chlamydospore production (Fichtner *et al*. 2006). In burley tobacco production systems, acidifying fertilisers containing nitrogen are generally recommended as a control measure for *T. basicola* (Harrison and Shew 2001). However, manipulation of soil pH and ionic concentrations to levels that suppress black root rot is not often a solution available for growers, as such levels are often not optimal for plant growth.

Temperature and inoculum density influence disease development in cotton. The optimum temperature for growth of the pathogen in culture is 20−30°C (Lucas 1955; Mauk and Hine 1988) and survival rates of *T. basicola* are greater at temperatures of 10−18°C than at 24−34°C (Papavizas and Lewis 1971). However, black root rot is worse at cooler temperatures of 16−20°C and is prevalent in temperatures ≤26°C (Mauk and Hine 1988; Rothrock 1992), presumably since the lower temperature is stressing the growing plant and favouring the pathogen (Lloyd and Lockwood 1963; Mauk and Hine 1988). There may be some adaptation of strains of *T. basicola* to regional conditions, with an isolate from peat in New Zealand having a temperature optimum of 22°C, compared with 27°C for a cotton isolate from Narrabri, Australia (Honess 1994). Nonetheless, the temperature at the time of planting can influence the severity of black root rot on any crop and it can be used as a method of controlling the disease. The damage to cotton has been shown to be particularly severe when there is an extended period of cool weather in the spring or if crops are planted too early, whereas planting when temperatures are higher can reduce severity even though inoculum levels of *T. basicola* may be higher (Rothrock 1992; Jhorar 2004).

Biotic factors can also influence the severity of black root rot caused by *T. basicola*. The use of biofumigation crops has been examined for control of black root rot in cotton. Incorporation of 'green manure' crops such as canola, mustard and woolly pod vetch (*Vicia villosa*) releases compounds that are toxic to soil pathogens. Australian trials have shown that biofumigation crops do not eradicate *T. basicola* but do reduce disease severity sufficiently to warrant their use (Nehl *et al*. 2000). The hydrolysis product of the dominant glucosinolates released from the roots of canola, 2-phenylethyl isothiocyanate, was also reported to suppress soil pathogens *in vitro*, including *Thielaviopsis* (Smith and Kirkegaard 2002). Hairy vetch has been used successfully in the USA as a biofumigant, planted as a winter cover crop to reduce the incidence of black root rot in the subsequent cotton crop (Rothrock and Kirkpatrick 1995). It was shown to reduce inoculum density of *T. basicola* and the severity of black root rot on cotton by as much as 60% (Candole and Rothrock 1998). Vetch degradation produces ammonium. High levels of ammonium are suppressive to *T. basicola*, probably because plants exposed to high levels of ammonium produce substances that are toxic to *T. basicola* (e.g. putrescine) (Candole and Rothrock 1998). Other reasons could include an increase in the soil pH by the ammonium released. Despite its

demonstrated potential, the capacity of hairy vetch to reverse severe infestation of *T. basicola* has not been proven in fields where cotton is cropped regularly in Australia (Nehl *et al*. 2004*a*).

Cruciferous plants are commonly used for biofumigation because they produce isothiocyanates, which inhibit *T. basicola* and other fungi. Biofumigation with mustards elicited a reduction in black root rot in cotton by up to 70% (Matthiessen and Kirkegaard 2006). The introduction of lucerne or corn stover to soil resulted in a decline in inoculum density of *T. basicola* and suppressed black root rot, possibly because organic amendments such as these support other organisms that act as antagonists to *T. basicola* (Papavizas 1968). This was also shown in cotton production system in Spain when growing sugar beet as the preceding crop followed by residue incorporation (Delgado *et al*. 2006). In this case, the black root rot suppressive effect was also dependent on nitrogen and iron levels in the cotton-growing soil.

Biocontrol options for use against crop diseases, based on microbial antagonism in the soil, are gradually increasing in number, and they have been reported in several reviews on plant growth promoting and disease-suppressive microorganisms. *Pseudomonas* species synthesise a variety of biocontrol compounds that can suppress root diseases. *Pseudomonas fluorescens* concentrations are higher in suppressive soils than in soils conducive to black root rot (Ramette *et al*. 2003), while the *P. fluorescens* strain CHAO has been shown to suppress black root rot caused by *T. basicola* in disease-conducive soils (Stutz *et al*. 1989), including natural soils in Switzerland, in the presence of sufficient amounts of iron (Keel *et al*. 1989; Defago *et al*. 1990). Mutations in *P. fluorescens* CHA0 that blocked the production of Phl, HCN (Laville *et al*. 1992) and pyoluteorin reduced the ability of these mutants to suppress black root rot (Ramette *et al*. 2003; Frapolli *et al*. 2010). Furanones, produced by soil-borne actinomycetes, fungi and bacteria, have shown antifungal activity against phytopathogenic fungi (Paulitz *et al*. 2000). *Pseudomonas aureofaciens* has been shown to produce 3-(1-hexenyl)-5-methyl-2-(5H)furanone, a compound with antifungal activity against *T. basicola.* This furanone has structural similarity to an antifungal furanone produced by the soil fungus *Trichoderma harzianum*, which has been used as a biocontrol agent against phytopathogenic fungi (Paulitz *et al*. 2000). *Streptomyces hygroscopicus* strain TA21 can reduce the incidence of black root rot by 85.3% in greenhouse experiments by inhibiting hyphal growth and reducing spore germination (Yi *et al*. 2010). Coating of seeds with fungal biocontrol agents is also proving to be an effective control measure against *T. basicola*. An example of this is the use of *Paenibacillus alvei* strain K-165, which is applied as a seed coat and then goes on to colonise the rhizosphere and soil, inhibiting root colonisation by *T. basicola* (Schoina *et al*. 2011).

Biological stimulation of antimicrobial production by cotton has also been demonstrated. Treatment of cottonseed with preparations of the fungus *Trichoderma virens* stimulated phytoalexin synthesis in the seedling roots, which was proved fungicidal to the root pathogen *Rhizoctonia solani* possibly due to the formation of hydrogen peroxide from the breakdown of the phytoalexin. It may prove an effective control treatment for cotton seedling diseases including black root rot (Stipanovic *et al*. 1992; Howell *et al*. 1998, 2000).

Conclusions

Black root rot has been recognised as a pandemic in Australian cotton for over a decade, with gradual increase in incidence and severity. There are few disease-management options available for growers for controlling black root rot in Australia (Table 1) and most are mainly based on cropping practices that are not always feasible due to climate and topography. No solution has yet been fully developed through plant breeding or transgenic cotton to control black root rot, and while some promising approaches are arising, further research is required before disease resistant cotton may be developed for commercialisation.

Understanding the biology and ecology of *T. basicola* and its interactions with its cotton host and other microorganisms in the vicinity of the plant is crucial for developing further strategies for controlling black root rot. Recent studies have developed molecular tools for detecting and studying *T. basicola* and its interactions with cotton (Coumans *et al*. 2009, 2010, 2011; Huang and Kang 2010; Pereg 2011) as well as for investigating its relationship with other microorganisms in the soil (L. Pereg, unpubl. data). Elucidating pathogenicity factors in *T. basicola* in order to develop new control measures would greatly benefit from sequencing the genome of this important plant pathogen.

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