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COTTON RESEARCH AND DEVELOPMENT CORPORATION



FINAL REPORT

OVERSEAS TRAVEL

**DEVELOP COLLABORATIVE LINKS WITH RESEARCHERS AND
EXAMINE HOW BLACK ROOT ROT IS CONTROLLED IN THE USA**

May 1999

DAVID B. NEHL



NSW AGRICULTURE

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Cotton Research and Development Corporation
FINAL REPORT
OVERSEAS TRAVEL

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TRAVEL DATES: 9th to 23rd May, 1999

OBJECTIVES OF TRAVEL:

- * To examine ways in which plant pathologists and practitioners control black root rot in cotton in the USA, including the use of biofumigation crops such as onion or hairy vetch, disinfestation of fields using summer flooding, protective fungicides used as seed dressings etc.
- * To gain information and a better understanding of the epidemiology of black root rot and the biology of the pathogen.
- * To develop collaborative research links with plant pathologists working on black root rot and other diseases of cotton in the USA.

ITINERARY

9 May	Narrabri - Sydney - Los Angeles - Bakersfield
10-11 May	Field trials and researchers at Bakersfield and Kern County
12 May	Bakersfield - Merced Field trials and researchers, Merced County Merced - Visalia
13 May	Visalia - Corcoran Commercial cotton farms, J.G. Boswell Co. Corcoran - Merced
14 May	Field trials and researchers, Merced County Merced - Santa Rosa
15-16 May	Free weekend
17 May	Santa Rosa - San Francisco - Dallas - Fayetteville, Arkansas
18 May	Fayetteville - Delta Research Station - Little Rock - Fayetteville Field trials and researchers
19 May	Plant Pathology Department, University of Arkansas
20 May	Fayetteville - Dallas - Tucson, Arizona Plant Pathology Department, University of Arizona
21 May	Plant Pathology Department, University of Arizona, Tucson.
21-23 May	Tucson - Los Angeles - Sydney - Narrabri

EXECUTIVE SUMMARY

In the regions visited in the USA, black root rot of cotton does not appear to be as severe as in Australia, probably because of two factors: the lighter soils in which cotton is grown in the USA are less conducive to the disease and long term rotations with non-host crops are practiced. It is also feasible that the strains of the black root rot pathogen, *Thielaviopsis basicola*, in Australia are more pathogenic than those in the USA but this possibility remains to be tested.

A high degree of host specificity exists among different strains of *T. basicola*. Therefore, while the pattern of geographic dispersal of black root rot in Australia is consistent with the introduction of a virulent pathogen, there is an alternative explanation: that continuous cotton monoculture has selected for virulence in a population that was previously endemic in cotton growing regions.

Fungicidal and biological seed treatments used in the USA do not control black root rot adequately in the field. The possibility of adverse interactions between insecticides and fungicides has been noted. The fungicide benomyl, although not used commercially, has shown some activity against *T. basicola*.

Summer flooding does not eradicate *T. basicola* and hence gives temporary control (up to four years of cotton). Summer flooding is constrained by topography and the availability of water, and is not widely practiced in the USA.

In the soils in Arkansas, biofumigation with woolly pod vetch gives a high degree of control against black root rot with positive economic returns. In California, rotation with onions and garlic is reported to give effective control. Rotation with sorghum can result in lower levels of black root rot than in continuous cotton but there is no evidence that this effect is any different to a bare fallow.

There is no evidence for resistance to black root rot in any cultivars or breeding lines of cultivated cottons. Resistance to *T. basicola* may potentially be found in wild species of cotton, possibly *Gossypium arboreum*. Transformation of cotton with genes for novel chemicals with antifungal activity is a potential source of resistance in future.

Root border cells can play a role in the susceptibility and resistance of plants to pathogens. In cotton, root border cells appear to act as decoys for *T. basicola* and prevent lethal infection of the vascular tissue via the root tip. The potential for enhanced mortality of cotton when *T. basicola* enters the vascular tissue through interaction with root knot nematode has been clearly established. Since *T. basicola* is now widely distributed in Australia the introduction or spread of nematodes (or any other pathogen that may enhance penetration of the vascular tissue of roots by *T. basicola*) could have dire consequences for cotton production.

Collaborative links were established with researchers in California, Arkansas and Arizona. The exchanges of information on black root rot research has provided new leads for development of a management strategy for black root rot in project DAN122C and helped avoid unnecessary duplication of research. Collaborative research will be continuing with Professor Rothrock and Professor Hawes.

RECOMMENDATIONS

- * The prospect for control of black root rot using contemporary fungicides as seed dressings is low and research emphasis should be shifted to investigation of in-furrow application.
- * The potential for phytotoxic interactions between fungicides (eg triadimenol) and insecticides (eg aldicarb) should be examined.
- * Summer flooding can be an effective control measure against black root rot but in Australia it will be limited by the availability of water and sites with flat topography.
- * The potential for long-term rotation (ie 3 to 4 years out of cotton) to reduce the severity of black root rot should be investigated.
- * Screening for sources of resistance in wild cottons (initiated in project DAN122C) should be continued.
- * Collaboration with researchers developing transgenic cottons with genes for novel antifungal agents should be sought, if and when this technology becomes available.
- * The role of root border cells in disease resistance in cotton should be investigated in collaboration with researchers at the Department of Plant Pathology at the University of Arizona.
- * Research on biofumigation should be expanded to include onions and/or other species of *Allium*.
- * Collaborative research on *T. basicola* with Dr Rothrock, Department of Plant Pathology at the University of Arkansas, should include investigations of the potential for development of decoy crops and studies of the effects of biofumigation on populations of *T. basicola*.
- * Researchers in Australia should utilise the TBCEN agar methods used at the University of Arkansas for isolation and enumeration of *T. basicola* from soil and plants.
- * Research on the pathogenicity and relatedness of *T. basicola* strains in Australia should be undertaken in collaboration with existing work at the University of Queensland, with comparison to strains of *T. basicola* from the USA.
- * The potential for interaction between *T. basicola* and the Fusarium wilt pathogen merits further investigation, particularly with respect to entry of vascular tissues by *T. basicola*.
- * Pathologists in Australia need to remain alert for the presence of plant pathogenic nematodes that may potentially interact with *T. basicola*. The potential damage to the cotton industry if incursions of quarantinable pathogens occur must be emphasised.

SIGNIFICANT ITEMS

California Planting Cotton Seed Distributors (CPCSD), Shafter

Dr Richard (Dick) Garber, consulting pathologist at CPCSD, provided a large amount of information about black root rot and associated seedling disease that is not readily available in the scientific literature or is unpublished, as follows.

- * Some strains of *T. basicola* can increase cotton growth in pots (apparently published by Cecil Yarwood cannot be located by abstract searches). The mechanism for this is not well understood. Yarwood's observations confirms the observations elsewhere that host specificity varies among strains of *T. basicola*.
- * The fungicide benomyl has some protective value against *T. basicola* in cotton grown in pots. Dr Richard Garber observed a decrease in disease index in cotton from the maximum 5.0 in untreated soil to 1.3 in benomyl treated soil. Some farmers have used benomyl as an in furrow spray (off label?) and suggest that it can be used to control *T. basicola*, although the practice is not current. A protective effect of benomyl against black root rot in tobacco has been reported (Saenger, 1970).
- * Unpublished work by David Ferguson and Dr Richard Garber showed a dramatically lower level of VAM infection in cotton grown in soil with a high population of *T. basicola* than in soil with a low level of *T. basicola*.
- * In the 1980's the virtual elimination of black root rot symptoms in cotton was observed in a section of a field where potatoes were planted previously. Black root rot was severe in the other half of the field that was planted to corn previously. This observation has not been confirmed in a replicated trial.
- * Dr Richard Garber has loaned a copy of the PhD thesis by E.J. Butterfield on Verticillium wilt in cotton during the early 1970's. In a trial in a field with severe Verticillium wilt, the incidence of black root rot and abundance of spores of *T. basicola* in the soil was four fold higher after continuous cotton than after rotation with sorghum. However, the effect of sorghum rotation was no different to that of a bare fallow, indicating that sorghum did not have a biofumigation effect. In the same experiment, consecutive irrigations over a six week period during mid-summer had no effect on disease caused by *T. basicola*.
- * Strains of *T. basicola* isolated from carrot tend not to be very pathogenic on cotton. Tabachnik has published work on this in Phytopathology.
- * There is a negative interaction between triadimenol (BaytanTM) and aldicarb (TemikTM). Establishment of cotton seed treated with Baytan was decreased 34 % by aldicarb at the Schuh ranch near Merced.
- * Current seed treatments recommended for control of *T. basicola* by California Planting Cotton Seed Distributors include:

Nu-Flow ND Nusan/Cloroneb + Apron + Nu-Flow M	(Wilbur/Ellis)
Vitavax-PCNB + Apron + Baytan	(Gustafson)

 Dr Richard Garber considers that Baytan does not give satisfactory control of black root rot.
- * Dr Garber has been evaluating seed coating formulations ("carriers") for some time. *Seed Biotics* in Idaho makes a calcium compound that is a cheap and reliable carrier. *Cepiril* has a product (Cepiril AI) that performs well on both fuzzy and delinted seed. *Colorcon* also has a good product for fuzzy and smooth seed which is the same material used to coat confectionary.
- * Rotation with onion or garlic apparently works well for control of *T. basicola* but is apparently not practiced.
- * Some lines (eg Maxxa, Royale) used in California used to have very good resistance to *Pythium* and appeared to tolerate black root rot to some extent. Over the years the resistance to *Pythium* seems to have eroded. (Dr James DeVay suggests that this may be due to dilution of the Maxxa genome during seed production). There is little evidence of resistance to black root rot in any upland or Pima germplasm in the USA.

In the Cotton Disease Council 1999 National Cottonseed Treatment Trial at CPCSD there was little black root rot present and black root rot was not assessed quantitatively. However, qualitative observations indicated that none of the fungicide used as seed treatments were effective against black root rot.

A field trial at Button Willow Land & Cattle Co. ranch (Figure 1) included two biological control agents selected by Dr Charlie Howell from College Station in Texas for activity against *T. basicola* in the laboratory. The level of black root rot was more severe at this trial site than at the CPCSD site (although less severe than levels commonly observed in Australian cotton). The two biocontrol agents appeared to have little activity against black root rot in the field at this trial site. Dr Howell apparently has further organisms that have greater activity against *T. basicola* in the laboratory but these organisms have not yet been tested in the field.



Figure 1. Dr Richard Garber inspecting a seedling disease control trial west of Shafter, California.

Black root rot was not present in a cotton variety trial at the Starrh ranch, east of Shafter. At this ranch cotton is rotated with lucerne (both crops irrigated), usually with a ratio of 4:4 years cotton:lucerne. Under this rotation they have had little black root rot so far, although the ranch is relatively new.

Fusarium wilt is restricted to about 70 to 75 farms in California and is not spreading further because of the resistance to nematodes in current cotton cultivars. Hence, fusarium wilt in cotton is no longer considered a problem in California.

Merced Field Station, University of California Davis, Merced

Dr Bill Weir is an extension officer with the University of California Davis and is based at Merced. Dr Weir has observed an interaction between aldicarb (Temik) and the fungicide triadimenol (BaytanTM). Dr Weir has observed smaller seedlings and a reduction in yield when both Temik and Baytan are used together, but not when used separately, in trials in Kern County (Bakersfield) and Tulare County. The interaction was apparently more evident in years when seasonal conditions were less favourable to cotton. The interaction had not been observed in Merced county prior to this season where it was observed in a trial with combinations of the fungicides Baytan and Nu-flow M and Temik at the Schuh ranch. On 13 May 1999 Dr Weir found that stand establishment in Baytan treated cotton was reduced 34 % by application of Temik. Dr Nehl and Dr Weir visited this trial on the previous day and observed black root rot but were unable to make any objective measurements due to an insecticide application.

The only sites visited in California where severe black root rot was observed by Dr Nehl were in Merced County at a number of cotton variety and seed dressing trials. These farms were on the western side of the San Joaquin Valley and had heavier soils than in the Bakersfield area (Kern County) where little black root rot was observed.

In a cotton variety trial at the San Juan ranch all lines appeared equally susceptible to *T. basicola*, sometimes with 100 % of the tap root showing the characteristic blackening of the root cortex (Figure 2).



Figure 2. Cotton seedlings with black root rot in a variety trial in California. Cultivars DP 6100 on the left and Maxxa on the right.

Black root rot severity was assessed quantitatively by Dr Nehl in a seedling trial run by Dr Weir with combinations of the product Nu-Flow M (marketed by Wilbur-Ellis). None of the Wilbur-Ellis seed treatments had a significant effect on black root rot severity or incidence (Table 1). The mean for severity of black root rot, 64 % of the tap root blackened, was lower than levels observed in fields in Australia with continuous cotton. However, black root rot was more severe near the tail ditch end of the field. The mean severity of black root rot in the buffer zone right at the tail ditch was 90 %, which is comparable to levels observed in fields with continuous cotton cropping in Australia.

Table 1. Severity of black root rot in cotton with various fungicidal seed treatments.

Treatment		Tap root blackened (%)	Plants infected (%)
Check plus		57	97
Nu-Flow M + Apron XL + Maxim plus	(1.75, 0.32, 0.08)*	64	98
Nu-Flow M + Apron XL + Nusan plus	(1.75, 0.32, 2.25)	62	100
Nu-Flow M + Apron XL + Nusan plus	(0.875, 0.16, 1.125)	69	98
Nu-Flow ND + Nu-Flow M + Apron XL plus	(14.5, 1.75, 0.32)	59	97
TCMTB + Maxim + Nu-Flow M + Apron XL plus	(2.25, 0.08, 1.75, 0.32)	63	100
Proized (Nuflow ND + Apron)		62	100
Nu-Flow M + Apron XL + Maxim	(1.75, 0.32, 0.08)	68	100
Nu-Flow M + Apron XL + Nusan	(1.75, 0.32, 2.25)	72	100
Nu-Flow ND + Nu-Flow M + Apron XL	(14.5, 1.75, 0.32)	72	100
TCMTB + Maxim + Nu-Flow M + Apron XL	(2.25, 0.08, 1.75, 0.32)	67	100
Untreated		59	95
		$p = 0.48$	$p = 0.50$
		$n = 8$	$n = 8$

* Rates in ounces per hundred weight of seed are in brackets.

Black root rot was assessed quantitatively by Dr Nehl in a second seedling trial run by Dr Weir with the same biological control treatments as in the trial observed in Kern County. In comparison to a control treatment, the two biological treatments did not reduce the severity of black root rot significantly (Table 2).

Table 2. Incidence and severity of black root rot in plots with and without biological control agents applied to the seed.

Treatment	Tap root blackened (%)	Plants infected (%)
Control	79	100
Treatment 1	86	100
Treatment 2	84	100
	Not significant	Not significant

Dr Weir recommends to growers that if crops have severe black root rot then irrigation should be timely to avoid water stress in the seedlings due to the damage to the root system.

J.G. Boswell Company, Corcoran, California

Dr Nehl visited the farms of J.G. Boswell Company at Corcoran and inspected the El Rico ranch with District Manager Mr George Wurzel. Summer flooding has been used at El Rico for some time to reduce the populations of seedling pathogens. Cost seems to be the main limiting factor to flooding as a control practice. The El Rico ranch is situated in the Tulare lake basin where the gradient is as low as 0.18 m fall per km. Flooding is usually done after wheat is stripped. The depth seems not to be important as long as the soil is covered for at least 30 days with diurnal temperatures of 29°C or more. Flooding is sometimes extended to two to three months, leading to good soil moisture for planting cotton the following spring. Occasionally flooding will bring salts to the soil surface if the field is drained during hot weather. Flooding doesn't appear to present a problem to VAM fungi in cotton. The reduced level of disease after flooding apparently lasts for approximately four years, after which flooding may need to be conducted again.

Dr Nehl visited two fields at El Rico that were in their second year of cotton following flooding, one field with Pima and one with upland cotton treated with Nu-Flow M. Black root rot was found in both crops. Overall severity of black root rot was not as high as in fields visited near Merced but up to 50% of the tap root blackened in some seedlings. Damping off was also observed. Clearly flooding did not eliminate seedling pathogens. Nu-Flow M is used on cotton seed at El Rico. Pima cotton is sown after flooding with no seed treatment at all, apparently achieving better results than when treated. Mr Wurzel considered that Nu-Flow M gave incomplete protection against *T. basicola*.

Plant Pathology Department, University of Arkansas, Fayetteville

Professor Craig Rothrock, Department of Plant Pathology, University of Arkansas has been researching black root rot in cotton for several years. Key points of interest arising from discussions with Dr Rothrock, his students and colleagues and examination of his field work include the following:

- * As in California, seedling disease appears to be a major concern in Arkansas. Dr Rothrock is developing a predictive model to enable farmers to use weather forecasts to decide whether or not to use in-furrow fungicides for seedling disease control. The model will be based on (i) the efficacy of in-furrow fungicides, using data from past and present field trials; (ii) soil temperature, using shading treatments prior to emergence to alter soil temperature; (iii) and rainfall, using irrigation to simulate precipitation events.



Figure 3. Dr David Nehl and Dr Craig Rothrock inspecting cotton seedlings in the Cotton Disease Council National Cottonseed Treatment Trial at the Delta Research Station.

- * In Ashley County (southern Arkansas) there appears to be an association between root knot nematodes and *T. basicola*. Fields with 100 propagules of *T. basicola* per g of soil are twice as likely to have threshold populations of *Meloidogyne incognita* as fields where *T. basicola* is not present. This observation may simply reflect the conduciveness of particular soils to both pathogens. An extensive survey of soil fertility and disease incidence is almost complete in Ashley County.
- * Dr Rothrock uses TBCEN agar for all isolations of *T. basicola* from both roots and soil. This medium contains carrot juice and can be used to produce propagules of *T. basicola*. Chains of chlamydospores can be separated into individual cells by treatment with chitinase.
- * Dr Rothrock has estimated yield loss due to *T. basicola* at 10 %. Seedlings were grown for some weeks in potted soil with and without inoculum of *T. basicola*, then transplanted to the field. Yield loss due to *Rhizoctonia* and *Pythium* was also measured at 10 % using the same method.
- * Woolly pod vetch (*Vicia villosa*) has been part of a long term green manure trial at the Delta Research Station on the western side of the Mississippi River (Figure 4). Cotton is grown every summer with either vetch or clover or a fallow during winter. The vetch is either drilled or broadcast. Since vetch provides 30 lb of nitrogen per acre (up to 100 lb per acre has been claimed) the cost of growing vetch (\$30 to \$40 per acre) is covered by the increased yield, reduced disease and N savings. Vetch typically reduces the incidence of black root rot from 35 % of plants to 5 to 8 %. Despite continuous cotton cropping, the severity of black root rot at the Delta Research Station appears, therefore, to be lower than in Australia where many fields have a 100 % incidence.



Figure 4. Seedling cotton at the Delta Research Station after a green manure of woolly pod vetch (LHS) or a winter fallow (RHS).

- * Vetch has been reported as a host for *T. basicola* but Dr Rothrock has not observed infection of vetch in his trials. Vetch is also a host for *Pythium* but does not seem to increase the level of *Pythium* infection in cotton. In Arkansas, vetch could act as a trap crop for nematodes. However, if it were allowed to grow into spring then the nematodes would complete a generation and cause a nematode problem.
- * The active biofumigant from growing vetch is ammonia gas released during breakdown of the vetch residues. To avoid phytotoxic effects on cotton, vetch should be incorporated at least three weeks prior to sowing. Dr Rothrock uses a portable instrument for *in situ* measurement of ammonia released when vetch is incorporated and will bring this instrument to Australia during his sabbatical at Narrabri. This instrument may possibly be adapted for *in situ* measurement if isothiocyanate production in biofumigation experiments in Australia.
- * Fungicides can have an adverse interaction with biocontrol agents used for seedling diseases of cotton. For example, Baytan can inhibit production of viridiol in the gliotoxin pathway in *Trichoderma virens* (*Gliocladium virens*); gliotoxin can be an inhibitor of pathogenic fungi. However, some of the biocontrol agents being developed for seedling diseases of cotton at College Station in Texas may also elicit systemic induced resistance.
- * Dr Fred Bourland, a Cotton Breeder at Delta Research Station, is evaluating resistance to *Verticillium* in cotton cultivars. He holds little hope for resistance to black root rot with current cotton cultivars.
- * Bronze wilt is becoming more of an issue. Symptoms of bronze wilt can't be successfully reproduced in the glasshouse and it does not necessarily occur in fields where it was severe in the previous season. Seed companies are discontinuing any cultivars with a tancot background because of the perceived problem with bronze wilt, even though many of those cultivars yield better than others when bronze wilt is not present. Growers in Arkansas have stopped growing these cultivars even though the disease was only seen for the first time in NE Arkansas in 1998 and in southern Arkansas in 1997. Growers and others have mis-diagnosed verticillium wilt as bronze wilt, even though there is no vascular discolouration in bronze wilt.
- * Dr Kirkpatrick, extension pathologist of University of Arkansas stationed at Little Rock, has observed that when the Bollgard gene was inserted into a line of cotton that was very resistant to root knot nematodes, in the process of backcrossing the breeders didn't screen for resistance to root knot nematode and it was lost. All the transformed plants died when nematodes were present in the soil.
- * Dr Terry Kirkpatrick first noticed an apparent interaction between *T. basicola* and root knot nematodes. In a field with both pathogens, there was almost a complete stand loss if Temik was not used. This work led to the doctoral research of Nathan Walker at the University of Arkansas.
- * Mr Nathan Walker has almost completed his doctoral research on the interaction between *T. basicola* and *Meloidogyne incognita*. Mr Walker used transparent root boxes to investigate these interactions and the technique should prove useful for research into black root rot in Australia. *T. basicola* affects the temperature range at which *Meloidogyne incognita* is pathogenic and vice versa.
- * *T. basicola* infects the cortex of cotton roots but does not kill the plant because it does not penetrate the endodermis or the vascular tissue. Until now, seedling mortality in soil infested with *T. basicola* was only known to occur in association with other seedling pathogens, namely *Rhizoctonia* or *Pythium*. Following Dr Kirkpatrick's observations in the field, Mr Walker has demonstrated that *T. basicola* will kill cotton

seedlings when root knot nematodes are present. The nematodes penetrate the root and disrupt the endodermis, enabling *T. basicola* to infect the vascular tissue. If nematodes are present in the soil then symptoms of vascular browning caused by *T. basicola* will occur in 80 to 95 % of plants. If nematodes are not present then the vascular tissue remains healthy. The interaction between *T. basicola* and nematodes has been confirmed in the field. Thus two pathogens that are non-lethal individually interact to cause seedling death.

- * After infection of the vascular tissue by *T. basicola*, the female nematodes either die because they are immobile at that stage of their life cycle or they change into males that are mobile. While the males are then able to leave the root, they cannot feed or change back to a female and will then die. So the interaction leads to an increase in propagules of *T. basicola* and a decrease in propagules of the nematodes. Fumigation to control nematodes consistently reduced the severity of black root rot in cotton and sporulation of the pathogen.
- * Some resistance to *Rhizoctonia* has been observed in two lines of *Gossypium arboreum* by Dr Gannaway at College Station in Texas and one of these lines has some resistance to *T. basicola*. Dr James (Mac) Stewart, of the Plant Pathology Department at the University of Arkansas, described a method whereby these resistance genes might be transferred to upland cotton.
- * Dr Stewart has also constructed a gene for magainin, an antifungal peptide of 23 amino acids that is produced by frogs. Magainin inhibits growth of *T. basicola* and Dr Stewart is working on transforming cotton with this gene for disease resistance.

Plant Pathology Department, University of Arizona, Tucson

Dr Martha Hawes has been working on the biology, both physical and molecular, of root border cells and their role in plant microbe interactions in the rhizosphere for many years. Discussions with Dr Hawes, her students and colleagues in the plant pathology department are summarised as follows:

- * Dr Mary Olsen, extension plant pathologist, indicated that in Arizona black root rot occurs only sporadically in fields where cotton is not rotated with other crops. When cotton is rotated with three to four years of lucerne the disease is not a problem. Rotation of cotton with wheat, 1:1, does not make black root rot worse but does not improve it either, as observed in Australia. There is some suggestion that rotation with sorghum or corn may reduce the severity of black root rot in cotton but this has not been proven experimentally.
- * Dr Martha Hawes, has shown that free-living root border cells express genes for susceptibility and resistance to pathogens in a similar manner to the cells of the root. A mutant strain of *Agrobacterium tumefaciens* that was chemotactically attracted to the root but not the border cells lost its ability to cause disease when the host was grown in soil. *Pythium dissotocum* is attracted to root border cells of cotton, infects them and kills them. However, *P. dissotocum* is not attracted to cotton roots if border cells are removed. Since *P. dissotocum* is widely present in cotton fields but does not cause disease in cotton, the border cells may act as decoys, preventing disease.
- * Dr Hawes has observed that infection of cotton roots by *T. basicola* always occurs above the root tip. She suggests that cotton border cells may act as a decoy to prevent infection of the root tip.
- * Uvini Gunawardena, PhD student, is investigating plant pathogen interactions at a molecular level, particularly with respect to root border cells. Border cell production increases in response to some pathogens. Border cells are actively involved in signalling between host and pathogen.

- * Xiao Wen, PhD student, has shown that exudates from border cells in some plant species repel nematode larvae and even cause temporary paralysis of nematodes.
- * Dr Lindy Brigham, has shown, that production of root border cells is determinate and can be switched on and off according to loss of border cells or presence of pathogens. Since the vascular wilt pathogens that affect cotton infect the roots at the root tip, border cells may play an important role in the resistance to *Verticillium dahliae* and *Fusarium oxysporum vasinfectum* shown by some cotton cultivars.
- * Dr Brigham has also investigated the role of substances involved in plant defences against soilborne pathogens. Some defence compounds (eg shikonen) are expressed or accumulate exclusively in root border cells.

The research on plant/pathogen signalling and differential expression of resistance genes in root border cells clearly has implications for the investigation and manipulation of resistance to soilborne pathogens in cotton. For example, the deployment of synthetic genes or genes from wild species in transgenic cotton may be enhanced, or dependent upon, localised expression of those genes in border cells.

Discussion of findings

Severity and distribution of black root rot

Black root rot was first recorded in Australia in 1989 and has since spread at an exponential rate and was present in 53% of the 90 fields examined in NSW in November 1998. In the USA the pathogen has been present for several decades and climatic conditions are conducive to the disease. It is notable, therefore, that in comparison to Australia black root rot appeared to be less common and less severe in the areas visited in the USA. At some farms near Merced (ie the highest latitude where cotton is grown in California) the severity of black root rot was comparable to that usually observed on Australian farms. However, black root rot was generally not severe at the sites visited in Arkansas and in the San Joaquin Valley near Bakersfield and Corcoran.

Given the rapid spread of black root rot in Australia, the incidence and severity of black root rot in California was not congruent with the length of time that the pathogen has been present. There are three possible explanations: i) the greater diversity in cropping practices in the San Joaquin valley (eg. 4:4 year cotton:lucerne rotation at Starrh ranch) may slow the spread and increase of inoculum; ii) the heavier soils in Australia may be more conducive to the disease; iii) Australian strains of *T. basicola* may be more virulent than those in California.

The pattern of geographic dispersal of black root rot in Australia has been consistent with the introduction of the pathogen to regions of previous absence. However, the pathogenicity of strains of *T. basicola* can be specific for the host from which they are isolated and not others. Since a variable degree of host specificity evidently exists within the genome of *T. basicola*, it is also possible that continuous cotton monoculture in Australia has selected for virulence in a population that was previously endemic in cotton growing regions. If such selection has occurred, then the genes for virulence would need to have been widely dispersed within the population, albeit at low levels. Research currently being undertaken at the University of Queensland may help answer this question.

Chemical control

The chemicals Nu-Flow M and Baytan are recommended seed treatments in California. While these chemicals do have a degree of activity against *T. basicola*, when they are used in seedling trials the major emphasis is on assessment of seedling mortality due to *Rhizoctonia* and *Pythium*. Seedling disease (ie stand loss caused by *Rhizoctonia* and *Pythium*) appears to be a greater problem to cotton production in the USA than in Australia. Since *T. basicola* does not cause seedling mortality by itself, the value of

fungicidal seed treatments for control of black root rot would be overlooked if only seedling mortality is assessed in fungicide trials. For example, Dr Weir has previously observed that Nu-flow M gives good control of black root rot. However, in the particular trial assessed by Dr Nehl and Dr Weir at Merced, the severity of black root rot was not significantly affected by a range of seed dressing treatments that included Nu-flow M. In general, the perceptions of growers and researchers are that Nu-flow M and Baytan give incomplete control of black root rot but that they are better than nothing.

The possibility of adverse interactions between insecticides and fungicides (eg Temik and BaytanTM) or for fungicides to interfere with biological control agents (eg. BaytanTM and *Trichoderma virens*) is evident in the USA.

The fungicide benomyl has shown some activity against black root rot in pot experiments but its effects in the field have not been examined quantitatively. Benomyl is worthy of further investigation under Australian conditions.

Biological control

So far, no biological agents with potential to control black root rot effectively have been developed in the USA. However, biocontrol agents for seedling disease caused by *Pythium* and *Rhizoctonia* continue to be evaluated and some will be screened for activity against *T. basicola*.

Biofumigation and crop rotation

Biofumigation with woolly pod vetch has been evaluated for some years now and is effective against black root rot in the soils near the Mississippi in Arkansas. In California there is good evidence that rotation with onions and garlic is effective against black root rot. Rotation with sorghum can result in lower levels of black root rot than in continuous cotton but there is no evidence that this effect was any different to a bare fallow. Therefore, since sorghum is not a host for *T. basicola*, the rotation merely delays the build up of inoculum observed with continuous cotton crops.

Host resistance

There is no evidence for resistance to black root rot in any cultivars or breeding lines of the four species of cultivated cottons. Resistance to *T. basicola* could potentially be found in wild species of cotton. The reported resistance to *T. basicola* in *Gossypium arboreum* lines is encouraging. Transfer of such genes would be a long term project but would ultimately offer the most effective control. The screening study initiated in DAN122C should be continued.

Novel chemicals with antifungal activity may provide an efficient method for control of black root rot. Of particular interest is a gene constructed for production of magainin, an antifungal peptide with demonstrated activity against *T. basicola*, that is currently being inserted into cotton.

Flooding

In California, summer flooding is not a viable control for black root rot in cotton anywhere other than the Tulare Lake area due to a lack of water and sloping ground. It is unlikely that flooding would have a wide application under Australian conditions. However, should seasonal conditions enable access to suitable quantities of water, then farms with low gradients may be able to experiment with this control measure. However, the economics of flooding should be weighed against the duration of control. Growers observed a useful level of control for only four years of cotton after flooding. Additionally, if flood banks are required within fields then a compaction problem would result.

Biology of black root rot

Root border cells can play a role in the susceptibility and resistance of plants to pathogens. In cotton, root border cells may act as decoys and prevent infection of the root

tip by *T. basicola*. Nathan Walker has demonstrated that *T. basicola* kills cotton seedlings if given the opportunity to enter the steele. Hence, if *T. basicola* were to infect the root tip and enter the vascular system, then plant mortality would be greatly increased. The role of root border cells in disease resistance in cotton merits further investigation.

The potential for enhanced mortality of cotton by *T. basicola* through interaction with root knot nematode has been clearly established. Since *T. basicola* is now widely distributed in Australia the introduction or spread of nematodes (or any other pathogen that may enhance penetration of the vascular tissue of roots by *T. basicola*) could have dire consequences for cotton production. Hence the possibility of interaction between the Fusarium wilt pathogen (*Fusarium oxysporum* f.sp. *vasinfectum*) and *T. basicola* should be investigated. Furthermore, quarantine measures should be maintained as a priority in Australia.

Collaboration

Collaborative links were established with researchers in California, Arkansas and Arizona. The exchange of information on black root rot research with Dr Richard Garber, Dr Bill Weir and Professor Rothrock has provided new leads for development of a management strategy for black root rot in project DAN122C and will help avoid unnecessary duplication of research.

Dr Weir and Dr Nehl have continued to exchange information since completion of the overseas travel. Dr Craig Rothrock will be visiting Australia for six months on Sabbatical leave during the second half of 1999. Dr Rothrock will be sharing his expertise in sampling for the fungus *T. basicola* and in sampling volatile compounds released in the soil during biofumigation, and will be collaborating with Dr Nehl on biofumigation and decoy cropping for control of black root rot. As a result of the discussions held in Arkansas, Dr Rothrock is now providing cultures for a student at the University of Queensland who is working on the genetic relatedness of strains of *T. basicola* in Australia.

Dr Martha Hawes and students at the University of Arizona will be involved in a collaborative investigation of the role of root border cells in pathogenesis in cotton, particularly in relation to black root rot, Verticillium wilt and Fusarium wilt. Arrangements have been made to provide seed of Australian cotton cultivars for this investigation.

Dr James McD. Stewart, University of Arkansas, has invited Dr Nehl to write a chapter on mycorrhizal symbiosis in cotton for the revised edition of *Cotton Physiology*.

Extension of results

On 31 May 1999, Dr Nehl presented a one hour seminar to researchers at the Australian Cotton Research Institute summarising the results of this research.

Researchers from DPI in Queensland have been alerted to the possibility of interaction between *T. basicola* and *F. oxysporum vasinfectum* and this issue will be discussed further at a meeting in August this year.

The technical officer from Bayer Australia Ltd who has been collaborating with field trials in project DAN122C using fungicides to control black root rot has been alerted to the possibility of a phytotoxic interaction between Baytan and insecticides.

FINANCIAL SUMMARY

All expenses were met from funds approved and allocated by the Cotton Research and Development Corporation. No other funds were available and Departmental approval for travel was given on the condition that there would be no cost to NSW Agriculture.

Item	Budget \$	Expenditure \$
Air Fares (Economy Class) Narrabri to Sydney to Los Angeles, San Francisco to Fayetteville to to Tucson to Los Angeles Return	2866.00	2785.30
Other Fares Hire-car, LA to San Francisco	450.00	513.23
Taxi/bus fares	80.00	0.00
Subsistence (show daily rates) 12 days @ \$95 (\$US60)	1140.00	
12.375 days @ \$US90 ('US other' rate)		1693.39
Other (specify) Accommodation 12 days @ \$110 (\$US70)	1320.00	585.24
Insurance	0.00	126.00
TOTAL	5856.00	5703.16