COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

OVERSEAS TRAVEL

ATTEND 8TH INTERNATIONAL CONGRESS OF PLANT PATHOLOGY, CHRISTCHURCH, NEW ZEALAND

February 2003

DAVID B. NEHL

NSW AGRICULTURE

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Cotton Research and Development Corporation

FINAL REPORT

OVERSEAS TRAVEL

RESEARCHER: **Dr David B. Nehl**

ORGANISATION: **NSW Agriculture**

POSITION: **Research Scientist**
Australian Cotton Research Institute
Narrabri NSW 2390

TELEPHONE: **02 6799 1528**
FACSIMILE: **02 6799 1503**

TRAVEL DATES: **28th Jan to 9th Feb, 1999**

OBJECTIVES OF TRAVEL:

* To participate in the 8th International Congress on Plant Pathology
* To present recent work for peer review at an international level
* To keep abreast of recent advances in plant pathology and integrated disease management practices.
* To maintain existing, and develop new, contacts and collaborations with the international community of plant pathologists, particularly with other plant pathologists working with cotton diseases and soilborne diseases.

ITINERARY

28 Jan	Orange - Sydney
29-30 Jan	Pre-conference workshop: *IXth International* Fusarium *Workshop*, University of Sydney
30 Jan	Sydney - Narrabri
2 Feb	Narrabri – Sydney - Christchurch
3-4 Feb	8th International Congress of Plant Pathology
5 Feb	8th International Congress of Plant Pathology, Australasian Plant Pathology Society Council Meeting Australasian Plant Pathology Society Biennial General Meeting
6-7 Feb	8th International Congress of Plant Pathology
8 Feb	Plant Health Australia Workshop: Developing a world class plant pathology diagnostic network Christchurch - Sydney
9 Feb	Sydney - Narrabri

EXECUTIVE SUMMARY

Dr David Nehl attended the 9th International Fusarium Workshop, the 8th International Congress of Plant Pathology and a workshop by Plant Health Australia on Developing a World Class Plant Pathology Diagnostic Network.

Outcomes

- New concepts in disease control and epidemiology are being incorporated in current research projects on diseases of cotton, including:
  - Biological soil disinfestation
  - Fumigation of soil using nitrogen fertiliser with nitrification inhibitors
  - Novel agents for induction of systemic acquired resistance, including nitric oxide and silicon compounds
  - Role of matric potential on reproduction of *F. oxysporum* f.sp. *vasinfectum* on crop residues

- Established new links and strengthened existing links with plant pathologists in the Ministry of Agriculture in Israel, at the University of Pretoria in South Africa, at the University of Arizona, at the University of Arkansas, at the University of Sydney, at the Royal Botanic Gardens in Sydney.

- Information on factors that may influence biological control of Fusarium wilt with non-pathogenic strains of *Fusarium oxysporum* communicated to Honours Student Christina Bakker at the University of Queensland.

- Work on a draft biosecurity plan for cotton in Australia has commenced in collaboration with Queensland DPI, Cotton Seed Distributors and the Australian Cotton Growers Research Association.

Outputs

- Report and recommendations to the Cotton R&D Corporation
- Two posters presentations
- One keynote paper (at Fusarium Workshop)
- One invited review paper in a refereed journal based upon work in the diseases of cotton project (see section 1.1)
- One oral presentation of highlights of the Congress given to peers at a NSW Agriculture Crop Protection Workshop

RECOMMENDATIONS

* An understanding of the pathogenicity and biogeography of pathogen populations, and the potential for gene flow within these populations, is essential for maintenance of effective disease management strategies and breeding for resistance. Continued study of the pathogenicity and genetic diversity of the pathogens causing Fusarium wilt, Verticillium wilt and black root rot in cotton in Australia is recommended.

* Investigations of the potential for biological control of Fusarium wilt of cotton using non-pathogenic *Fusarium oxysporum* should consider the role of root exudates in the disease suppression and factors affecting root exudation (e.g. mycorrhizal fungi).

* Future deployment of diagnostic tests for prediction of disease severity in cotton may be compromised by pathogens that have high competitive saprophytic ability (eg. *Rhizoctonia*, *F. oxysporum* f.sp. *vasinfectum*) and the influence of climatic conditions on disease development. These factors should be accounted for in development of integrated disease management strategies.

* Novel activators of systemic acquired resistance should be investigated, including silicon compounds, nitric oxide, fungal metabolites and lactofen.

* Non-pathogenic strains of soilborne pathogens (e.g. *Fusarium oxysporum*) have potential for biological control of disease and should be investigated, particularly in the context of field soils and root exudates.

* The effect of dryness of cotton residues on the reproduction of *Fusarium oxysporum* f.sp. *vasinfectum* should be investigated.

* Novel practices for disinfestation of soil, including biological disinfestation of soil and organic amendments combined with nitrification inhibitors, should be examined for their potential to control of soilborne pathogens of cotton.

* The potential for suppression of vascular wilts in cotton by sudan grass (and associated fungi) should be investigated in field experiments.

* The potential for aerial dispersal of *F. oxysporum* f.sp. *vasinfectum* in Australian cotton needs to be investigated.

* Pathologists in Australia need to remain alert for the presence of plant pathogenic nematodes that may potentially interact with *T. basicola* in cotton. The potential damage to the cotton industry if incursions of quarantinable pathogens occur must be emphasised.

SIGNIFICANT ITEMS

IXth International Fusarium Workshop

Dr Kerry O'Donnell (USDA, Peoria, Illinois) discussed recent advances in understanding of phylogenetic relationships of strains within *Fusarium graminearum*, causing head blight of wheat. *F. graminearum* actually consists of 9 different clades that should be considered as biogeographically distinct species. Dr O'Donnell stressed that ribosomal genes were not that useful for phylogenetic analysis of species of *Fusarium*. Dr R. Bowden, (USDA, Manhattan, Kansas) extolled the virtues of AFLP markers for phylogenetic studies of *F. graminearum*. The capacity to distinguish these different species of *F. graminearum* has profound implications for development of disease resistance. For example, head blight in the USA is predominantly caused by lineage 7, in Mexico lineage 3, in China lineage 6, and in Australia lineages 2, 7 and 8. Hence, the resistance incorporated in cereal and maize lines that are bred in one region, may not be effective in other regions of the world.

Dr Cees Waalwijk (Plant Research International, Netherlands) reported the changing spectrum of species associated with Fusarium head blight in western Europe. PCR diagnostic tests were used to distinguish species of *Fusarium*. In the 1990's *Fusarium culmorum* was dominant but in the early 2000's it was *F. graminearum*.

Dr R. Dill-Mackay (University of Minnesota) described integrated disease management strategies for Fusarium head blight, using host resistance, chemical and biological controls, and cultural methods.

Dr Toshiaki Takehara (National Agricultural Research Centre, Tsukaba, Japan) described biological control of Fusarium wilt in spinach, caused by *Fusarium oxysporum* f.sp. *spinaciae*, by a non-pathogenic *Fusarium oxysporum* (NPF) strain. Competition for nutrients appeared govern the suppressive effect of the NPF on Fusarium wilt and, therefore, root exudates probably play a role in the capacity for suppression. The suppression was effective in soils where natural depletion of glucose was rapid.

Dr David Backhouses (University of New England) discussed factors affecting crown rot of wheat. Differences in disease severity in one year do not affect disease severity in subsequent years because the pathogen survives saprophytically and climatic conditions have a major influence on disease development. This has implications for the usefulness of diagnostic tests for saprophytic pathogens (eg. a similar scenario exists with *Rhizoctonia*, causing seedling disease on cotton).

Dr Naresh Magan (Cranfield University, UK) reported that the influence of soil matric potential on reproduction of *Fusarium* is more important than that of solute potential. The implication for control of Fusarium wilt of cotton is that dryness of cotton residues may affect reproduction of *F. oxysporum* f.sp. *vasinfectum*.

Dr Jaacov Katan (Hebrew University of Jerusalem) reported that *Fusarium oxysporum* f.sp. *vasinfectum* in cotton in Israel produces macroconidia, on cotton stems, that have potential for aerial dispersal. The potential for aerial dispersal of *F. oxysporum* f.sp. *vasinfectum* in Australian cotton needs to be investigated.

Dr Julie Flood (CABI, UK) described the resurgence of Fusarium wilt of coffee, caused by *Fusarium xylarioides*, in west and central Africa. This disease was a problem during the 1950's and 1960's but was overcome by breeding for resistance. Virulent new strains of *F. xylarioides* are now causing devastating losses (e.g. 10 million trees killed in Uganda since 1993). Mature trees can die within 2-6 weeks of infection. It is not known if this pathogen is seedborne but measures to prevent its spread to Australia would be prudent.

Dr John Leslie (Kansas State University) stressed that validation is the most important issue for molecular diagnostics. In particular, the number of strains used to develop the test is important and should be determined using collections from field populations, not strains in "fridge" collections.

Dr David Nehl (NSW Agriculture) reviewed the epidemiology of Fusarium wilt of cotton in Australia and the current research effort aimed at developing integrated disease management.

8th International Congress of Plant Pathology

Cultural methods

Dr Jaacov Katan (Hebrew University of Jerusalem) described the development of solarisation for disease control. Hurdles for the use of solarisation include climate suitability, the time for which land is tied up (3 to 6 weeks) and heat tolerant pathogens (eg. *Macrophomina*). The advantages of solarisation include induced suppressiveness to pathogens, wide spectrum disease control (greater spectrum than methyl bromide), reduced use of biocides, and it's long term effects. Solarisation can be enhanced when combined with amendment of soil with chicken manure (see Gamliel and Stapleton, 1993).

Dr J. G. Lamers (Applied Plant Research, The Netherlands) discussed the novel technique of biological disinfestation of soil. In this method, green manure crops are incorporated into the soil, the soil is irrigated and then covered with plastic sheeting that has low permeability to oxygen. This creates anaerobic conditions and, if conducted for six to 10 weeks during warm conditions (i.e. summer), gives good control of *Verticillium dahliae* and *Fusarium oxysporum*.

Dr George Lazarovits (Agriculture and Agri-food Canada, London, Ontario) described novel methods to control Verticillium with soil amendments that release ammonia. Blood and bone amendment (2%) resulted in total eradication of *Verticillium dahliae* in some soils, partial eradication in other soils, and had no effect in others. Nitrification inhibitors can enhance this effect by blocking nitrification, which results in a build up of ammonia. The activity of the ammonia is better at 5% soil moisture content than at 10%. The efficacy of suppression increases as pH increases from 7 to 8.5. If the pH of manure is

changed from 7.7 to 5.0 the effect is enhanced and this can be achieved by adding sulphuric acid to the manure. This method has potential for eradication of soilborne pathogens of cotton in Australia, which is mainly grown on alkaline clays soils.

Dr Tony Pattison (Queensland DPI, Innisfail) presented results of biofumigation with brassicas to control nematodes in banana. Several of the species that have been tested for biofumigation potential against soilborne fungal pathogens of cotton in NSW, were also effective against nematodes.

Prof. Randal Row (Ohio State University) discussed integrated management of Verticillium wilt in potato. Good control of the disease was achieved after three years of green manure crops (i.e. a biofumigation effect). The pathogen is spread in seed-potato but this inoculum does not colonise plants as fast as first thought. Soilborne inoculum may be just as important. Jim Davis in Idaho showed that green manures with sudan grass gave control of the disease. *Fusarium equiseti* is associated with sudan grass and this fungus could be involved in the suppression of Verticillium wilt.

Quarantine issues

Lester Burgess (University of Sydney) discussed the importance of risk assessment and area freedom.

Prof. Craig Rothrock (University of Arkansas) described crop losses in cotton due to roo knot nematode. This pathogen was able to cause up to a 31% yield loss over two years. The need for continued quarantine barriers against nematodes that are pathogenic on cotton is paramount.

Prior to the workshop on Developing a World Class Plant Pathology Diagnostic Network (see below) the development of a biosecurity plan for cotton in Australia was discussed with Dr Simon McKirdy (Plant Health Australia).

Population genetics

Prof. Bruce McDonald (Swiss Federal Institute of Technology) presented a keynote paper on using the genetic diversity of pathogens to improve disease management. If the pathogen population has high diversity and low gene flow, then major genes for host resistance and quantitative resistance must be deployed. If the pathogen population has high diversity and high rates of gene flow the quantitative resistance is the only option. If the pathogen population has low diversity and high rates of gene flow, then gene pyramids can be used because clones of the pathogen are not likely to exchange genes. If the pathogen population has low diversity and low rates of gene flow then single major genes for resistance will be effective. Hence, it is important to understand the nature of the pathogen population to develop a resistance strategy.

Dr Dolores Gonzalez (Departmenta de Systemática Vegetal, Xalapa, Mexico) reported that *Rhizoctonia solani* is not monophyletic and needs to be re-named and split into different species.

Dr K.V. Subbarao (USDA) discussed pathogenic groupings within *Verticillium dahliae*. Originally there were 15 vegetative compatibility groups (VCG's) (see Joaquin and Rowe, 1991) and these were collapsed to 4 VCG's (Strausburgh et al. 1993). Lorolev et al. (1997, 1999) added sub groups. Baht and Subbarao (2003) found that there was no correlation between VCGs and pathogenicity on bell pepper. It is not clear whether or not *V. dahliae* can be considered as one breeding population. Many new hosts have succumbed to *V. dahliae* in the past decade, indicating plasticity in the host range of this pathogen. However, it was not expected that lettuce would become a host, as is now the case. Important questions include: is the origin of variation in *V. dahliae* related to the intensity of agriculture and the diversity of crops in a given area?; Is the soil population the same as that isolated from different hosts? More information on the origin of pathogenic variation in the field and the basis of host resistance is required.

The genus *Verticillium* has be revised (see Gams, 2002, Mycological Research 106: 129-131).

Dr Katherine Dobinson (Agriculture and Agri-food, Canada) discussed genomic approaches to understanding the pathogenicity of *V. dahliae*. The parasexual cycle, melanin synthesis and VCGs are well understood but there is little genetic information available on this fungus. Searchable databases such as COGENE have potential to aid understanding of this pathogen.

Prof. Ian Crute (Rothamsted) discussed the potential for genomics to result in healthier crops. Genomic models have progressed in some areas and not in others. The genome of *Arabidopsis* is well understood and this information will become public knowledge. However, while there have been advances in understanding of viral pathogens, little is known about the genomes of bacterial and fungal pathogens. 'Comparative' genomics is required to advance understanding of plant pathogens. For example, the Broadbalk experiment was conducted for 150 years at Rothamstead, during which time yields increased 300%, yet in the same period we still have not achieved sustainable management of late blight. Publicly funded advances in knowledge are required, independent of commercial interests. A raised level of public sector funding will require some collective vision. Plant pathogens continue to evolve and this evolution is a constraint to sustainability (e.g. fungicides will not last forever). The most effective gene for resistance will only work in one crop with one disease in one region. Therefore we need to exploit genes that in plants that make them resistant to most pathogens (multi-pathogen resistance).

Biological control

Prof. B. Gerhardson (Swedish University of Agricultural Sciences) described biological control of seedborne fungal infections in cereals by inoculation of the seed with the bacterium *Pseudomonas chloraphis*. The most important factor in the success of *P. chloraphis* is it colonisation of the plant and survival under field conditions. Placement of the bacterium between the embryo and the endosperm was optimal and 100 cells per seed could multiply to 10^6 cells per seed if this placement was achieved.

Dr. Genevieve Defago (Swiss Federal Institute of Technology) discussed how soil suppressiveness can be specific to one pathogen, with several hosts. In a study by Rameth et al. (2003) there were no differences in levels of *Pseudomonas* between soils that were suppressive and conducive to black root rot on tobacco. In other studies, the expression of biocontrol-genes in *Pseudomonas* was greater in the presence of a non-pathogenic *Fusarium*. However, fusaric acid may counteract the effectiveness of biological control agents. Collembola may increase the effectiveness of *Trichoderma* as a biocontrol agent in wheat. These studies indicate the complexity of soil community dynamics.

Dr Claude Allabouvette (University of Bourgogne, France) discussed factors involved associated with soils that are suppressive to Fusarium wilt. Biological control of Fusarium wilt is difficult because it is hard to prevent the pathogen from penetrating the root and it is protected once inside the root. Yet in suppressive soils there is a low incidence of disease despite the presence of the pathogen. Most suppression involves microorganisms. Work with *Pseudomonas* spp. and non-pathogenic *Fusarium oxysporum* (NPFo) indicated that the NPFo completes with the pathogen for carbon. When *Pseudomonas* spp. are also applied, they compete for iron, which enhances the competition for carbon. The *Pseudomonas* colonised the same sites as the pathogenic *Fusarium oxysporum* and reduced saprophytic growth of the pathogen on the root surface. Both the NPFo and the *Pseudomonas* can activate induced systemic resistance (ISR). The ISR was sufficient to reduce disease when the *Pseudomonas* and the pathogen were inoculated onto either side of a split-root system. However, the NPFo was not able to reduce in the split-root system, which indicated that competition was the major factor in biological control of disease with the NPFo. Host responses induced by the NPFo include bi-phasic release of extracellular peroxide, increased rate of Ca^{++} efflux, and increased rates of apoptosis (programmed cell death, analogous to the hypersensitive response). Mutants of NPFo can be more or less effective as biological control agents, indicating the multigenic nature of the disease suppression.

Dr David Weller (USDA, Pullman, Washington) discussed disease decline. Take-all decline is transferable from a suppressive to a conducive soil (with as little as a 1% soil amendment) and this effect is eliminated by pasteurisation (even a mild pasteurisation at 60°C for 30 min). The pseudomonads involved in the decline all contain Ph1D genes and produce the antibiotic DAPG (2,4-diacetylphloroglucinol). The population of DAPG-producing pseudomonads in take-all decline soils is generally greater than 10^5 cfu/g root and their population density is inversely related to the severity of take-all. DAPG-producing pseudomonads are also associated with suppression of Fusarium wilt in pea and in flax. Seventeen genotypes of DAPG-producing pseudomonads have been distinguished using repPCR (now using RFLP of ph1D gene) and these genotypes are predictive of biological activity in soil. The D genotype is an aggressive coloniser of pea. DAPG-producers are not effective against *Rhizoctonia*. Strains of the D genotype transformed with a gene for phenazine (antibiotic) have been produced but are unable to displace parental strains in the field.

Dr Harry Hoitink (Ohio State University) discussed manipulation of microbial communities for disease suppression. In over 100 batches of compost, less than 3% activated Induced Systemic Resistance (ISR) in plants. Dr Hoitink suggested that composts could be inoculated with bacteria that activate ISR.

Dr Stephen Neate (North Dakota State University) described disease decline of *Rhizoctonia* in cereals. The disease decline is unlikely to be due to a single organism yet the literature usually reports research on single organisms. Greater understanding of the interactions between different organisms associated with disease decline is required before soils can be managed to exploit this phenomenon.

Disease Resistance

Dr Thorsten Nürnberger (Institute of Plant Biochemistry, Germany) discussed signal perception in plant defence responses. Plants are able to recognise pathogen-associated molecular patterns (PAMPs) and these appear to play a role in plant defences.

Dr Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Germany) discussed mechanisms of non-host resistance to fungal pathogens of plants. *Arabidopsis* mutants, at PEN loci, allow penetration of epidermal cells by the barley pathogen *Blumeria graminis* f.sp. *hordei*. Haustoria form after penetration but the plant develops an HR-like response anyway. The PEN genes are thought to play a role in defence responses, anchoring vesicles to the plasmalemma at the sites of cell wall apposition adjacent to the invading penetration peg. Hence PEN genes don not react directly on the cell wall. PEN genes are important because in a compatible disease reaction the fungus may need to elicit responses in the papilla. There is some evidence that PEN genes are active in plant roots and they seem to have an effect with *Fusarium* but this has not been tested with a soilborne pathogen.

Prof. Matteo Lorito (Universita degli Studi di Napoli Fedrico II, Italy) reviewed candidates genetically modifying plants to provide or enhance disease resistance, including: antifungal genes from mycoparasitic fungi, avirulence factors, inducers of systemic acquired resistance, receptors that recognise the pathogen, and pathogen inducible promoters.

Dr Helen McFadden (CSIRO, Canberra) discussed transgenic approaches to control of vascular wilt fungi. Defensins appear to be more promising than others. In tobacco transformed with the antifungal peptide magainin, which was expressed in the chloroplasts, leaf extracts suppressed *V. dahliae*. It may be possible to increase the antifungal activity of these peptides by creating synthetic antifungal peptides by mutagenesis. Similarly, it may be possible to create better genes for resistance through mutagenesis. Conversely, fungal mutants can be utilised to study infection processes in plant-pathogen interactions.

Dr Bart Geraats (University of Utrecht, The Netherlands) showed that when tobacco plants were transformed with a gene (Tetr) from *Arabidopisis* that made them insensitive to ethylene, the became more susceptible to *Pythium*

and *Thielaviopsis basicola* and *Fusarium*. This indicates that ethylene has a role in the infection process.

Induced resistance

Dr Harry Hoitink (Ohio State University) discussed manipulation of microbial communities for disease suppression. In over 100 batches of compost, less than 3% activated Induced Systemic Resistance (ISR) in plants (see Hoitink and Boehm, 1999, in Annual Review of Plant Pathology, 37: 427-446). Dr Hoitink suggested that composts could be inoculated with bacteria that activate ISR.

Prof. Richard Belanger (University Laval, Canada) discussed defence responses associated with induced resistance. Plant flavonoids were induced in large quantities in cucumber by silicon compounds and acted as a phytoalexin against powdery mildew. The fungus itself does not induce these flavonoids.

Prof. Ray Hammerschmidt (Michigan State University) discussed practical applications of induced resistance. It is possible that fungicides (e.g. metalaxyl, prophenazole, phosphonate) may induce resistance, plus some herbicides, especially lactofen.

Dr D. F. Klessig (Boyce Thompson Institute for Plant Research, Ithica, NY) discussed the multifaceted role of salicylic acid in induced resistance, including inhibition of catalase and ascorbate peroxidase (two major peroxide-scavenging enzymes). Salicylic acid binding protein (SABP) also plays a role in the defence response, which is downgraded if SABPs are silenced (e.g. silencing SABP2 in tobacco prevents induction of pathogenisis related protein 1 (PR1) by both SA and tobacco mosaic virus). Nitric oxide also inhibits catalase and ascorbate peroxidase activity. Nitric oxide synthase is induced by some plant pathogens and many enzymes that are associated with innate immune and inflammatory responses in mammals are also expressed in plants.

Prof. Eric Lamb (Rutgers University, USA) discussed the hypersensitive response (HR) and defence coordination. (see diagram in paper by Jane Glazebrook, 2001). Reactive oxygen species are common signals for programmed cell death (apoptosis) in eukaryotes. Ambient oxygen levels are required for induction of the HR not for induction of systemic acquired resistance (SAR). While induction of the HR requires ambient levels of oxygen, progression of the HR does not. Meta caspases, similar to the caspases associated with mammalian apoptosis, were recently shown to be associated with the HR in plants. Caspase-like activity is induced in plants by nitric oxide.

Dr Marty Dickman (University of Nebraska, USA) discussed signal transduction associated with fungal pathogens. *Colletotrichum trifolii* produces cutinases, that release plant cutin constituents, which in turn induce expression of lipid activated protein kinase (LIPK) in *C. trifolii*, which is important in formation of appressoria. It may be possible to exploit our understanding of host-pathogen interactions to improve the performance of agents that induce SAR in cotton in Australia, or to develop novel inducing agents (e.g. extracts from pathogens).

Dr Kim Plummer (HortResearch, New Zealand) discussed defence responses in apple to *Venturia inequalis*. This fungus secretes a protein *in vitro* that is an elicitor of the *Vm* resistance gene in apple. This suggests the possibility of using metabolites from pathogens to induce SAR in cotton.

Integrated disease management

Dr Ian Porter (Agriculture Victoria) discussed integrated management of club root in brassicas in Victoria, including farm hygiene, disease prediction, monitoring of pathogen populations, and precision application of calcium oxide.

Prof. Mike Jeger (Wye Imperial College, UK) discussed tactical and policy approaches to disease management. Comprehensive information on epidemics is required to understand epidemics well enough to manage them. Transgenic disease resistance is currently limited to resistance against viruses and is yet to be widely deployed. Pest risk analysis and risk mitigation is required to cope with biological invasions.

Australasian Plant Pathology Society

As a member of the Management Committee of the Australasian Plant Pathology Society (APPS), Dr D. Nehl participated in the APPS Council Meeting on 5th February.

Dr D. Nehl also participated in the APPS Biennial General Meeting on 5th February and moved that the Society establish a committee to actively confer with the South African Plant Pathology Society and other regional societies to develop a closer relationship and linkages with APPS. D. Nehl to confer with David Guest.

Workshop: Developing a World Class Plant Pathology Diagnostic Network

This workshop was convened by Plant Health Australia with the objective of developing a strategic plan to establish a national network of diagnostic laboratories within a quality assurance (QA) framework.

Dr Bill Roberts (AFFA) discussed the need for a diagnostic network. Key issues include:

- Competition between laboratories
- Limited coverage of pathogens and pests
- Few accepted protocols for diagnosis
- No agreed national funding mechanism
- Many thousands of pests/pathogens on over 300 crops in Australia
- Flexibility and the ability to act quickly are required
- The skills base needs to be maintained
- There is a lack of taxonomists
- Funding is sufficient but needs to be shared equitably
- Physical facilities are adequate but duplication needs to be minimised
- Bio-security facilities are not available for exotic organisms, largely due to industry nervousness
- DNA technology is promising but requires validation

- Multiple technologies need to be developed
- National policy is lacking and needs to address the following conflicts:
  - Public versus private good
  - Industry versus State versus Federal
  - Production versus exotic pest detection versus barrier control
  - Equitable cost sharing
- Standards are not enforced (anyone can set up a laboratory with no quality assurance)

Solutions to these issues include:

- Development of a national policy
- Development of collaborative arrangements with overseas laboratories
- Capacity building at the centre of origin of the pathogen (e.g. Karnal bunt would require linkages with India)

Dr Laurene Levy (USDA) described the American situation. There have been several recent incursions of exotic pathogens in the USA. For some there has been a conspicuous failure in diagnosis, due to lack of familiarity with symptoms and complacency, including misdiagnosis on the assumption that the pathogen was absent. A National Pest and Disease Diagnostic Network is now being developed. Discussion included the possibility of an international diagnostic network, legislation for compulsory notification, confidentiality of fee-for-service diagnostics, the need for recognition of patterns in epidemiology that aid diagnosis.

Dr John Elphenstone (Central Science Laboratory, UK) discussed the role of emerging technology in disease diagnosis. Real-time PCR will now enable serious application of diagnostics beyond niche areas. High throughput testing is now possible (e.g. up to 30,000 pathogens can be screened on one slide). On-site testing with portable real-time PCR is also available.

Dr Suzy Bentley (Queensland DPI) discussed issues related to modern diagnostic tests, including:

- Detection of organisms must be distinguished from diagnosis of disease
- Tests need to be rapid and definite for quarantine purposes
- Lack of familiarity with pathogen diversity limits the usefulness of available tests (e.g. tests may not be specific for strains)
- Cross specificity between strains is a confounding factor
- Tests need to be adapted to local conditions
- Monitoring is problematic
- Sampling has a number of issues, including: the type of sample; the need for specimens; specimen preservation (the test is only as good as the sample!)
- Validation must be done under Australian conditions
- Databases and collections are required

Discussion included: compliance with submission of samples (Dr D. Nehl); the need for networking nationally and overseas; the need to distinguish between absence and lack of records for a pathogen; the critical need for early detection of incursions; the need for a central high-security laboratory (and their high cost) versus sending researchers overseas; lack of human resources in classical virology and anti-sera techniques; lack of taxonomic descriptions

limiting the capacity of new techniques to address all risks; the need for field staff to conduct systematic sampling with central diagnosis.

Prof. Denis McGee (Iowa State University) described the National Seed Health System in the USA. Scientific input to this system has been critical, including peer review and accreditation.

Dr Richard Sheldrake (NSW Agriculture) discussed resource management and funding challenges for plant diagnostics, including the following issues:

- The need to identify clearly what is required and thus avoid under-allocation or over-allocation of funds
- The restrictions of our GDP on the available budget for diagnostics
- The need for governments to provide services in the areas that the community wants
- Competing demands for government funding
- The need for: defined outcomes; laboratory efficiency; training and competency; contractual arrangements; quality control and accreditation

Discussion included: can Australia be self sufficient?; Government is a mirror of society and September 11 may change attitudes (e.g. bioterrorism); the need to distinguish between detection and diagnostics (D. Nehl); the need to get PCR diagnostics into the field.

Dr Margaret Williams (Tasmanian DPI) described the processes involved in accreditation of diagnostic laboratories. Rodney Turner (Plant Health Australia) discussed accreditation and training requirements.

There was broad consensus at the meeting for the need to have a comprehensive Australian diagnostic network, that recognises the needs of the clients that it serves. Key initiatives should include:

- Developing a cost-effective network for detection of exotic and existing pathogens by: enhancing the existing resources; identifying key centres/capabilities; and securing roles and responsibilities
- Implementing quality assurance and accreditation

Collaboration

Collaborative links were established with researchers in the USA, Israel and Australia. Fungicides used to control of seedling diseases in cotton in CRDC-funded project DAN154C were discussed with Frank Shotkoski (Syngenta Biotechnology Inc., North Carolina). The exchange of information systemic acquired resistance with Dr Elizabeth Dann has provided new leads for development of SAR as a control measure for diseases in cotton and will assist CRDC-funded projects DAN153C, DAN154C, and DAN176C. Prior and future collaborations with Prof. Craig Rothrock and Prof. Terry Kirkpatrick (University of Arkansas) were strengthened, with PhD Student John Harvey planning to visit Prof. Rothrock for collaborative research on black root rot of cotton in 2004.

Extension of results

On 25 March, 2003, Dr D. Nehl presented a 15 minute summary of highlights of the 8th ICPP to researchers at the NSW Agriculture Crop Protection Forum at Head Office in Orange.

PUBLICATIONS

Oral papers and poster abstracts

Nehl, D. B., Allen, S. J. & Kochman, J. K. (2003). Fusarium wilt of cotton in Australia: a fatal fungal affliction? In *IXth International Fusarium Workshop - Abstracts* University of Sydney: Sydney. (Oral paper)

Nehl, D. B., Allen, S. J. & Lonergan, P. A. (2003). Soilborne pathogens of cotton in Australia: threats and potential threats. In *8th International Congress of Plant Pathology* vol. pp. 332. Ed., International Society for Plant Pathology: Christchurch. (Poster)

Harvey, J. A., Nehl, D. B. & Aitken, E. A. (2003). Geographical distribution of *Thielaviopsis basicola* in Australia. In *8th International Congress of Plant Pathology* vol. pp. 258. International Society for Plant Pathology: Christchurch. (Poster)

Publications

David Nehl was invited to submit a refereed paper in *Microbiology Australia* based on the oral presentation at the IXth International Fusarium Workshop. (see Appendix)

Nehl, D. B., Allen, S. J. & Kochman, J. K. (2003 in press). Fusarium wilt of cotton in Australia: a fatal fungal affliction? *Microbiology Australia* **42**, 8-11.

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 Christchurch Return	1100.00	966.10
Orange to Sydney to Narrabri	Nil	337.10
Other Fares		
Taxi/bus fares	Nil	102.21
Subsistence(actuals)		
Accommodation, meals, incidentals	1995.00	1047.46
Other (specify)		
ICCP registration	970.00	982.20
Fusarium Workshop registration	450.00	409.09
Insurance	0.00	100.00
Medical exam	Nil	42.00
TOTAL	4515.00	3986.16

APPENDIX – INVITED PAPER, *MICROBIOLOGY AUSTRALIA*

Fusarium wilt of cotton: a fatal fungal affliction?

David Nehl[1,2], *Stephen Allen*[1,3], *Joseph Kochman*[1,4]
[1]Australian Cotton Cooperative Research Centre, Narrabri NSW Australia
[2]NSW Agriculture, Locked Bag 1000, Narrabri NSW 2390, Australia
[3]Cotton Seed Distributors Ltd, PO Box 117, Wee Waa NSW 2388, Australia
[4]Queensland Department of Primary Industries, PO Box 102, Toowoomba QLD 4350, Australia

Introduction

Fusarium wilt is one of those uncommon phenomena where something so small, does so much and motivates so many. A virulent, new fungal pathogen is afflicting a vibrant modern industry in the Australian rural landscape. In this review, we examine the appearance of Fusarium wilt of cotton and the substantial response by the researchers and farming communities behind Australia's third biggest rural export commodity.

The nature of the beast

Fusarium oxysporum Schlecht. is a cosmopolitan fungus causing vascular wilts in many agricultural plants. Fusarium wilt of cotton (*Gossypium hirsutum* L.) is caused by *F. oxysporum* f.sp. *vasinfectum* (Atk.) Snyd. and Hans. (hereafter ***Fov***) and is specific to cotton. The disease was first confirmed in cotton in Australia on the Darling Downs of south east Queensland in 1993[1]. Two strains have since been distinguished on the basis of vegetative compatibility groups and DNA fingerprinting[2, 3]. It was recognised at an early stage that the strains in Australia were different to those affecting cotton overseas[2]. Recent evidence indicates that the Australian strains of *Fov* are most closely related to a single lineage of *F. oxysporum* occurring on native Australian species of *Gossypium*[4]. Continuous monoculture of cotton is likely to have played a role in selecting virulent members of the native population of *F. oxysporum*.

The Australian strains of *Fov* are highly virulent on all commercial cotton cultivars. *Fov* infects cotton at all stages of crop growth and the mortality of seedlings is frequently greater than 50%. *Fov* infects the cotton roots near the growing tip and then penetrates the vascular tissue. Once inside the vascular tissue, microconidia are produced abundantly and move rapidly up the xylem vessels with the flow of sap. The plant produces gums and tyloses (balloon-like structures) in an effort to occlude the vessels and limit the progress of the fungus. Occlusion of vessels is a 'suicide' strategy because the plant essentially 'blocks its own plumbing'. Symptoms in older cotton plants include vascular discolouration, foliar chlorosis and necrosis, stunted growth, wilting, plant death (Figure 1) and yield losses. Unlike some of the strains of *Fov* affecting cotton in the USA, which require co-infection by plant pathogenic nematodes before serious disease develops, the Australian strains are aggressive autonomous pathogens.

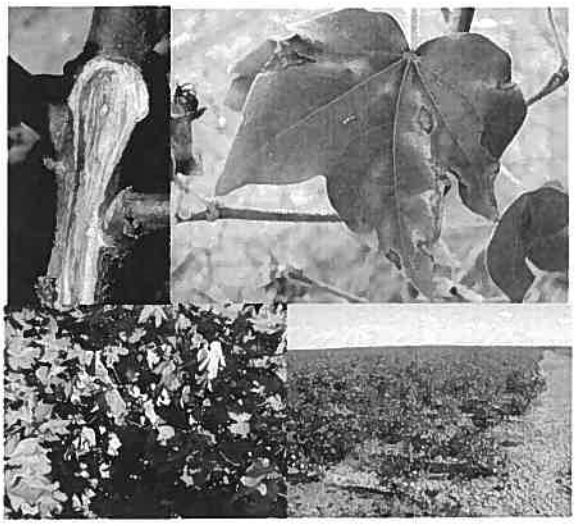

Figure 1. Symptoms of Fusarium wilt in cotton include vascular discolouration, foliar chlorosis and necrosis, wilting and death The Fusarium wilt epidemic

Fov has a high competitive saprophytic ability, enabling it to maintain inoculum levels in the soil for periods in excess of 10 years in the absence of a susceptible host plant[5]. The fungus survives as mycelium and chlamydospores in infested crop residues and soil. Hence, it is easily dispersed by irrigation, floodwater and the movement of soil and cotton trash adhering to vehicles and machinery.

After the initial outbreak was diagnosed in 1993 it was soon realised that it was too late for quarantine measures to prevent further spread of the pathogen. Symptoms of Fusarium wilt do not develop below threshold levels of inoculum[6]. Hence, the pathogen is usually present in the soil for a number of years before the population increases sufficiently for symptoms to be noticed. Fusarium wilt is now widespread in most cotton growing areas in NSW and Queensland. Surveys of cotton diseases, conducted annually by NSW Agriculture since 1984, indicate the classic exponential increase of an epidemic (Figure 2). If the pathogen continues to spread at the same rate, it is likely more than 90% of cotton farms will be affected by 2010 (Figure 2b). This fungus clearly threatens the profitability and sustainability of the Australian cotton industry and has elicited a large response in industry-funded research and control programs.

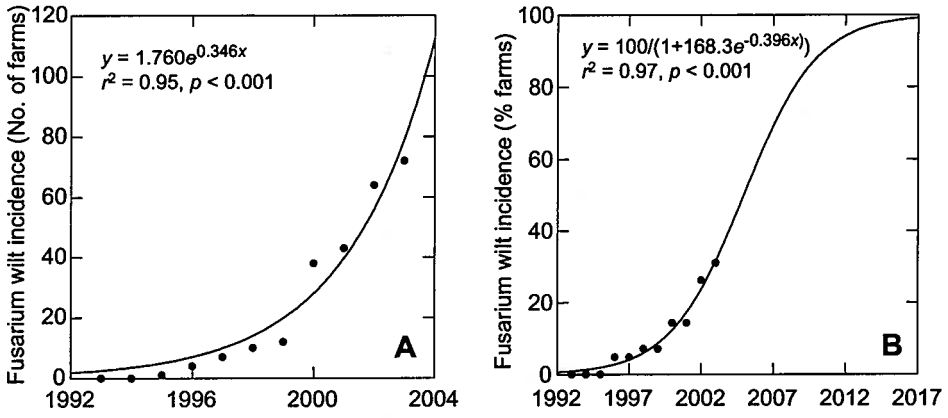

Figure 2. Historical and predicted increase in the incidence of Fusarium wilt of cotton in NSW (A: tally of all confirmed cases, B: proportion of confirmed cases in a subset of farms across NSW that are surveyed annually)

Integrated disease management

The ideal control for any plant disease is long-lasting multigenic resistance in the host. CSIRO Plant Industry and the commercial seed company DeltaPine Australia Ltd have both responded to Fusarium wilt with a sustained intensive breeding program. Although some promising germplasm is being developed, cultivars with a high level of resistance against *Fov* will not be available for some years. Current commercial cultivars are either susceptible or, at best, less-susceptible. We have monitored cotton crops in fields infested with *Fov* for a number of years. Planting the less-susceptible cultivars reduces the incidence of disease initially and slows the rate of increase. Due to the lack of highly effective host resistance, the cotton industry is deploying all available control measures in an integrated disease management approach.

From the outset a program of farm hygiene has been promoted to minimise the movement of *Fov* in infested soil or crop residues carried on vehicles, machinery, tools and even shoes. Marketed under the slogan "come clean – go clean", this program has been widely accepted by farmers and their communities. All are encouraged to remove adhering soil and debris as they leave any farm, thus ensuring "clean" arrival at the next. Many farmers have voluntarily installed facilities for this purpose (Figure 3). A very effective protocol to prevent dispersal of *Fov* in planting seed was also developed and implemented by the cotton industry.

Figure 3. An industry-wide farm hygiene program has been implemented to minimise spread of *Fusarium oxysporum* f.sp. *vasinfectum*, including "wash down" facilities exiting infested farms.

Efforts to break the 'chain of infection' by rotating with non-host crops have been frustrated by the ability of *Fov* to grow saprophytically on residues from a wide range of plants. Rotation of infested fields with wheat and barley can increase the severity of Fusarium wilt in subsequent cotton crops[7], while summer crops such as sorghum and maize appear to maintain the fungus in the soil.

A novel form of rotation cropping is biofumigation, where a non-host crop is ploughed into the soil as a 'green manure', with subsequent release of volatiles that are toxic to

soilborne pathogens. Biofumigation offers a safe, self-generating method of distributing a natural fumigant throughout the soil profile. Candidates for biofumigation include *Brassica* spp., that release isothiocyanates[8], and hairy vetch (*Vicia villosa* Roth) which releases ammonia[9]. Hairy vetch and brassicas have been used successfully for biofumigation against the soilborne fungus *Thielaviopsis basicola*(Berk. & Broome) Ferraris, which causes black root rot in cotton[9, 10]. In contrast, when hairy vetch, canola (Brassica napus L.) and mustard (Brassica juncea L.) were tested for biofumigation potential against *Fov*, the severity of Fusarium wilt in the subsequent cotton crop was increased dramatically in comparison to fallow soil (Table 1). Clearly there are pitfalls in extrapolating control measures from one pathogen to another.

Table 1. Increased severity of Fusarium wilt in cotton following incorporation of winter crops into the soil

	Winter crop	Healthy[z] cotton (plants ha^{-1})
Experiment 1[y]	Fallow	10600a
	Hairy vetch	1250b
	Indian mustard	1040b
		$p \leq 0.001$
Experiment 2	Fallow	6460a
	Canola	1560b
		$p = 0.003$

[z]Plants were classed as healthy when vascular discolouration was either absent or occurring in less than 5% of the stem cross-section near ground level.

[y]Values followed by the same letter are not significantly different by pairwise comparison of means with the Scheffé test at the stated probability.

Although the apparent potential for *Fov* to multiply on organic matter in the soil limits the options for crop rotation, there is potential to reduce the survival of *Fov* in cotton fields. Compared to immediate incorporation, leaving cotton trash to weather on the soil surface for 30 days after harvest decreased the severity of Fusarium wilt in the next cotton crop by 31%[7]. Flooding fields for 30 to 60 days during a summer fallow can dramatically reduce the severity of soilborne diseases of cotton[11]. We have observed the same with Fusarium wilt but the practicality of this solution is severely curtailed by the availability of water and the topography of fields[7].

All plants have inherent systems of defence against pathogens, although they are very distinct from the immune systems of higher animals. Using biological or chemical agents, it is possible to manipulate these defences in advance of the pathogen to produce a heightened state of resistance[12, 13]. Treatment of cotton with a non-fungicidal chemical (acibenzolar-S-methyl, Syngenta) can reduce the severity of Fusarium wilt[7]. We are currently evaluating the potential for seed treatment with acibenzolar-S-methyl to control Fusarium wilt. Although this induced resistance will not eliminate the disease, it appears to have potential as a component of an integrated disease management strategy.

Conclusions

The Fusarium wilt fungus is a virulent plant pathogen with a conspicuous capacity for survival and dispersal in space and time. As with most plant diseases, Fusarium wilt of cotton is exacerbated by the activities of man. Repetitive monoculture of cotton ensures that inoculum levels increase while intensive mechanised farming systems enable widespread movement of infested soil and crop residues. Despite a lack of effective tools to control Fusarium wilt, a concerted research and breeding effort is making

headway in developing an integrated disease management strategy involving farm hygiene, pathogen free planting seed, breeding for resistance, cultural practices and other novel controls. The fatal effect of Fusarium wilt on cotton has translated to a spirited and substantial response by the cotton industry.

Acknowledgements

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