Report for CSP92C

Travel to 7th Internation Verticillium Conference

I attended the 7th International Verticillium Conference, held in Greece from 6th-10th October, 1997. I was invited to present a keynote paper giving an overview of the propects for the use of genetically engineered crops for control of vascular wilt diseases. In addition I presented a paper describing experimental results obtained with transgenic cotton plants expressing either glucose oxidase or chitinase. These two presentations were submitted as papers for inclusion in the published Conference Proceedings. A detailed report on the scientific results presented at the meeeting is attached. This report will be circulated to colleagues via the Covert newsletter. Various valuable contacts were renewed (with Jane Robb, University of Guelph; and Kathy Dobinson, Agriculture Canada) and new contacts made with opportunities for collaborative research emerging. I will collaborate with Richard Cooper (University of Bath) to investigate the role of sulphur in tolerance of cotton to Verticillium wilt and with Oen Huisman (University of California, Berkeley) to use fluorescent antibodies to study Verticillium infection of transgenic cotton. I may be able to obtain constructs for generation of Verticillium expressing green fluorescent protein from Mary Powelson (Oregon State University).

When I was in England I visited Dr Tom Locke at ADAS Rosenmaund, Herefordshire. He is involved in assessing Verticillium load in soil as a basis for advising farmers about the suitability of various crops. ADAS is also coordinating an education/public awareness program aimed at increasing consumer and grower acceptance of transgenic crops in England.

Overall I found the trip to be very valuable for the exchange of scientific information and generation of contacts and collaboration. The generous funding support of the CRDC is gratefully acknowledged.

Report on 7th International Verticillium Conference,

Sounion, Greece, 6-10th October, 1997

attended by Helen McFadden, CSIRO Plant Industry.

The first sessions of this meeting concentrated on defining and characterising the species within the genus Verticillium. Jim Heale (King's College, London) gave an overview of Verticillium species, Milton Typas (University of Athens) summarised work using molecular marker technologies and Talma Katan (Volcani Centre, Israel) gave an overview of work on vegetative compatibility groups.

In Verticillium there are four plant-pathogenic species

V. albo atrum	alfalfa pathotype
	non-alfalfa pathotype
V. dahliae	cotton defoliating strains
	mint strains
	Italian clover strains
	Tomato strains, race 1 (controlled by Ve gene)
	race 2 (virulent on Ve+ hosts)
	Solanaceae strains (groups A, B, C and D)
V. tricorpus	essentially benign
V. longisporum	- a new species previously described as a variant of *V. dahliae*, pathogenic on Cruciferae including *Arabidopsis*.

Various marker technologies were described and applied to studies of variation in Verticillium species. RAPD analysis was found to be of limited use; results varying with isolation protocols and other factors. Use was made of fingerprinting using a dispersed repetitive DNA sequence (E18 probe) and PCR-RFLP analysis using primers for several mitochondrial DNA genes (cytochrome oxidase, NADH dehydrogenase and ssRNA). Using 4-base cutters *Hpa*II, *Cfo*I and *Hae*III, fingerprints allowing discrimination between 7 species was achieved. Several studies based on the use of PCR amplification of ribosomal RNA gene ITS regions, a technique developed in Jane Robb's laboratory (University of Guelph), were described. The use of HPLC to quantify the PCR products was found to give better linearity for quantification of fungal biomass. Skimmed milk powder was used to treat crude soil extracts to remove substances inhibiting the PCR reaction allowing detection of Verticillium species in soil samples. The method has also been extended to allow detection of various nematodes involved with Verticillium in potato early dying syndrome.

In order to test vegetative incompatibility groups (VGC's), isolates of Verticillium are grown on minimal medium containing potassium chlorate to induce nitrate- non-utilising mutants (*nit1* or *nit3* mutants). Mutants that can't utilise hypoxanthine as N source are also identified (nitM mutants) and then pairs of *nit1*/nitM mutants from the original isolate that readily form heterokaryons exhibiting wild-type growth on minimal medium containing nitrate are identified. These mutants are then tested for

heterokaryon formation against a set of tester strains (an international standard set for *V. dahliae* is available, Randy Rowe, Ohio State University). There are 5 VGC's recognised for *V. dahliae*, of which 1, 2 and 4 are most common.

VCG1A - defoliating cotton strains
VCG2A - non-defoliating, severe
VCG2B - " (cotton strains are severe on cotton, mild on eggplant)
VCG4A - milder, potato strains in NW USA
VCG4B - milder, potato strains in NW USA

Seven Australian cotton Verticillium isolates were tested using this system and all were found to belong to VCG4B (Talma Katan, Volcani Centre, Israel).

One session of the meeting was devoted to analysis and detection of microsclerotia. Aad Temorshuisen (Wageningen) presented the results of an interlaboratory study into methods for quantification of microsclerotia in soil. Highly variable results were obtained by different laboratories, highlighting the need for standardisation of methods in this field.

In the session concerning plant/pathogen interactions, Richard Cooper (Bath University) gave an overview of some interactions important in resistance and pathogenicity in Verticillium infections. Several enzymes are important in pathogenicity, eg. polygalacturonidase, lyase and pit-degrading enzymes. Attempts to study the effects of these enzymes on fungal pathogenicity using gene knockout experiments have been hampered by the presence of multiple isoforms of most of the enzymes. Treatment of tomato leaves with pectic enzymes can induce wilt-like symptoms. Wilt symptoms are due mostly to water stress caused by deposition of polysaccharides in the xylem vessels. Introduction of high molecular weight dextrans into xylem also gives wilt symptoms.

Verticillium can be present in the vascular system of plants without causing symptoms, but the symptom-inducing factors have yet to be identified. The vascular environment is liquid; in liquid culture Verticillium forms bud cells, whereas in solid culture and soil it grows hyphae. In infected plants, transpiration rates are reduced, even when other symptoms are not present. In defoliating Verticillium strains, plants produce elevated levels of ethylene and abscissic acid. The defoliating effect can be inhibited using silver thiosulphate to inhibit ethylene production, but other symptoms (wilt, leaf necrosis, vascular browning) are not affected.

Preformed resistance in the host may be due to the presence of saponins, phenolics and other molecules. The endodermis is extremely effective at preventing the movement of Verticillium from the root cortex into the xylem tissue. Conifers don't get Verticillium wilt, perhaps because of their short tracheids. Vessel ends slow down the spread of Verticillium through the xylem, so short xylem vessels may be associated with resistance. Induced resistance mechanisms incluse the formation of tyloses (bumpy outgrowths of parenchyma cells into the xylem to prevent pathogen movement in xylem vessels) and callose deposition. The parenchyma cells produce hemicellulose and pectin which occlude the vessels and contain the pathogen. Tolerant cultivars usually produce more phytoalexins, (including elemental sulphur) more rapidly than tolerant

plants. The possible mechanisms for formation of sulphur in xylem gels, vessel walls and parenchyma cells remain a mystery.

Helen McFadden (CSIRO, Canberra) presented an overview of prospects for the use of genetically engineered resistance to fungal pathogens. Promising results were obtained with transgenic plants expressing either glucose oxidase or chitinase. Transient expression of bacterial blight avirulence genes in cotton leaves containing blight resistance genes was shown to elicit an artificial hypersensitive response, demonstrating the potential for the use of controlled expression of avirulence genes as triggers of the host's endogenous defence responses. Several papers describing the performance of different crop cultivars under Verticillium pressure were presented. Infected petioles were identified as an important source of infection in tree nurseries, and Verticillium-contaminated seed potatoes (even from plants without symptoms) were implicated as a source of Verticillium potato early dying syndrome in NW USA and Canada.

Oen Huisman (University California, Berkley) presented an overview of the ecology of Verticillium infection. Observations of Verticillium infection were performed using fluorescent Verticillium-specific antibodies. Verticillium is a relatively slow-growing fungus in soil and is not fast enough to catch a growing root tip. Infection therefore occurs behind the root tip from the zone of elongation to a few mm behind this. Verticillium hyphae then grow in the cortex for several days and the bulk of fungal biomass is found in the cortex 4-8 cm behind the root tip. Only 1 in 4000 infections of the cortex successfully penetrated into the xylem. Microsclerotia are formed as aggregates that gradually disperse in soil. Thus the number of colony forming units in soil increases over a two-year period before gradually declining. The inoculum level is a function of formation rate + dispersal rate - death rate. Thus attempting to reduce Verticillium inoculum level by rotation in alternate years will not be successful. A simple model predicts that 6-year rotations are required. Hosts that support systemic infection contribute most to inoculum levels, because microsclerotia are produced most abundantly in aerial tissues. Potatoes give the greatest buildup of inoculum levels. When microsclerotia are present at 5-10 per g soil, every potato plant in a field is infected.

Control of Verticillium is frequently achieved using fumigation with methyl bromide. However, this chemical is due to be withdrawn, so alternative control measures are being sought. Promising results with various biocontrol agents, such as *Talaromyces flavus* (Debora Fravel, Beltsville, USA) *Trichoderma viride, Verticillium tricorpus* (Jim Davis, University of Idaho) and various bacterial antagonists (Eris Tjamos, University of Athens) were reported. These are probably most likely to be effective in the field when used in combination with other control measures. In some soils, use of organic amendments achieved spectacular control (George Lazarovitz, Agriculture Canada). However, the scale of amendments (green manure, soymeal, blood and bone meal) used at 18-30 tons per acre (!) is not economically viable. Work is in progress on achieving results with lower levels of treatment. The treatment is thought to work by release of gaseous ammonia . Some control was also achieved in warm climates using solarisation (covering the soil with plastic). Combination of organic amendments and plastic mulching may also be effective.

Results that emerged in general discussion were reported by Mary Powelson (Oregon State University) who is testing transgenic potato lines from Monsanto (transgene unidentified) and finding they exhibit good control of potato early dying syndrome. A worker in her laboratory has generated transgenic Verticillium expressing green fluorescent protein. Kathy Dobinson (Agriculture, Canada), who published a procedure for transformation of Verticillium, found that she could not obtain stable GUS expression in Verticillium. This confirms results obtained in our laboratory (CSIRO). A Monsanto researcher was reported (George Lazarovitz) to have presented promising results with antifungal peptides in transgenic potatoes at a Canadian Phytopathological Society Meeting in Winnipeg last June. Avi Nachmais (Gilat Experiment Station, Israel) claimed to have attempted the transfer of the tomato *Ve* gene from tomato to potato using protoplast fusion. he reported that the "gene" was located on several chromosomes, and expression of a gene product in the resulting potato plants was not detected. Further details were not available.

On the field trip to Livanates we observed Verticillium infection in olives, eggplant and cotton. Field trial of various biocontrol methods were in progress.