ATTACHMENT C

PROGRESS REPORT

Type of Application: FINAL REPORT

Corporation's Code: CSP52C

Genetic Manipulation of Natural Plant Defences for the Control of Verticillium wilt Disease in Cotton

Progress Report January 1997-August, 1977

Helen McFadden

The aim of this project is to generate and evaluate transgenic cotton plants that are expected to have improved tolerance to fungal disease, in particular *Verticillium* and *Fusarium* wilts.

Glucose oxidase. In order to confirm the results obtained previously, a second glasshouse trial was performed to test the tolerance of transgenic glucose oxidase-expressing plants to *Verticillium dahliae*. The results of this trial confirmed that glucose oxidase expression does give some protection against the severity of stunting and, at low inoculum levels, the incidence of symptoms due to *Verticillium* infection. However, because of deleterious effects associated with glucose oxidase expression (reduced germination rates, delayed root development, and reduced boll size in later bolls) even when using a root-specific promoter, it is not likely that the transgenic lines tested will provide the basis for commercially useful material. It may be possible, by use of more specific promoters, to target the protective effect so that useful resistance without associated deleterious phenotypes is obtained. A paper describing the biological effects of glucose oxidase in transgenic plants has been prepared (1).

Antifungal proteins. Transgenic cotton plants expressing chitinase have been generated. Elevated chitinase activity has been confirmed by enzyme assay of transgenic plant extracts. Homozygous T3 seed for the highest-expressing line has been generated. In an initial

glasshouse trial testing the *Verticillium* tolerance of this transgenic, chitinase-expressing line, significant reduction in the severity of *Verticillium*-induced stunting was observed. A second trial to confirm this observation is currently underway. Several chitinase-expressing lines are also being evaluated using enzyme assays to determine the relative levels of chitinase expression. Southern analysis is also being performed to determine the number of transgene inserts in each line. The results obtained with glucose oxidase and chitinase-expressing cotton plants were presented at the 7th International Verticillium Conference (Greece, Oct 6-10th, 1977). A written version of the paper was submitted for inclusion in the published conference proceedings (2). An overview of the potential applications of transgene technology for generation of host plant resistance to Verticillium wilt was also presented as an invited keynote lecture at this meeting (3) and submitted for publication in the conference proceedings.

Transgenic lines of cotton transformed with osmotin have been obtained. Generation of homozygous seed from one osmotin RNA-expressing line (identified by Northern analysis) for *Verticillium* testing is underway. In order to facilitate testing of possible synergy between osmotin and chitinase, crossing of osmotin-expressing and chitinase-expressing plants has been performed. Seed should be available for harvest from these plants in a few weeks.

No expression of β-1,3-glucanase was detected in NPTII-expressing (kanamycin resistant) cotton plants. Therefore a different construct for β-1,3-glucanase expression was synthesised by Rob de Feyter. Cotton transformation with this construct has been initiated. We anticipate that synergy between chitinase and osmotin may generate sufficient *Verticillium* tolerance for commercial application. No transgene expression was detected in selected plants transformed with truncated osmotin, truncated chitinase or acidic chitinase constructs. Further analysis of these lines has been suspended for the moment in favour of further work with the identified chitinase and osmotin-expressing material.

A recent publication demonstrated a synergy between a chitinase and Bt toxin against *Spodoptera sp.* It is therefore possible that chitinase enhances the activity of Bt toxin against

Helicoverpa sp. in transgenic plants. A preliminary feeding trial (conducted by Bill James, Division of Entomology) using transgenic chitinase-expressing cotton leaves demonstrated toxicity to *Helicoverpa* sp. Further testing of the insecticidal properties of this material, both alone and in combination with Bt, is underway.

Work on the above topics (except glucose oxidase) is continuing (Grant CSP86C).

In order to investigate the interaction of *Xanthomonas campestris* pv *malvacearum* (bacterial blight) avirulence genes with cotton blight resistance genes in the cotton cell, a series of constructs for avirulence gene expression was generated. Transient expression of avirulence genes in cotton leaves was achieved using *Agrobacterium* inoculation of mature cotton leaves. It was demonstrated that bacterial avirulence genes are expressed in the plant cell, and the products of the avirulence genes interact, in a gene-specific manner, with blight resistance genes. The interaction is characterised by an artificial hypersensitive response, demonstrating that avirulence genes have potential applications as triggers of plant defence responses in transgenic plants. A publication describing this work has been prepared (4). Further work using avirulence genes to trigger endogenous plant defence responses is underway (Grant CSP84C).

Publications

(1) Murray, F., Llewellyn, D., McFadden, H., Last, D., Dennis, E.S. and Peacock, W.J. (submitted to Divisional Panel). Expression of the *Talaromyces flavus* Glucose Oxidase Gene in Cotton and Tobacco gives some protection against Fungal Infection, but Exhibits a Phytotoxic Phenotype, for submission to **Molecular Breeding.**

(2) McFadden, H., de Feyter, R., Murray, F., Grover, A., Llewellyn, D., Dennis, E., and Peacock, W.J. (submitted). Genetic Engineering Approaches to the Improvement of Cotton's Tolerance to *Verticillium* Wilt. **Proceedings, 7th International Verticillium Conference, Greece, Oct 6-10th, 1997.**

(3) McFadden, H. (submitted). Prospects for Controlling Vascular Wilt Diseases of Cotton and other Crops by Genetic Engineering. **Proceedings, 7th International Verticillium Conference, Greece, Oct 6-10th, 1997.**

(4) De Feyter, R., McFadden, H., and Dennis, L. (submitted to Divisional Panel). Five avirulence genes from *Xanthomonas campestris* pv. *malvacearum* cause genotype-specific cell death when expressed transiently in cotton, for submission to **Molecular Plant-Microbe Interactions.**

Personnel

Helen McFadden (Principal Researcher)
Rob de Feyter (Co-worker and Co-Principal Researcher, New Grants, some CSD funding)
Danny Llewellyn (Sub-program Leader)
Liz Dennis (Program Leader)
Robin Chapple (Technician, new appointment under new grants)
Bill James (Collaboration with *Helicoverpa* trials, Division of Entomology)