with the physiological state of the cells in a callus which is in turn dependent on the effects of plant hormones. Furthermore, grafted crown gall-derived plants appeared as susceptible to virus infection as untransformed grafted plants. However, crown gall cells in many ways resemble meristematic cells and it is an intriguing possibility that part of the reason why many plants can be freed from virus by culturing apical meristems is due to the induction of the same resistance in these tissues as that associated with the presence of PRs in leaves.

The work described here was done in collaboration with G. Ooms and R.F. White (Biochemistry and Plant Pathology Departments, Rothamsted Experimental Station), and with G.J. Wullems and L. van Vloten-Doting (Department of Biochemistry, University of Leiden, the Netherlands).

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Pathogenesis-related-protein synthesis in selected cultivars of beans and cowpeas following leaf damage by carborundum, treatment with aspirin, infection with tobacco mosaic virus, or with the bean or cowpea strain of southern bean mosaic virus

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As an adjunct to a recently initiated, collaborative research project entitled 'The interaction of southern bean mosaic virus (SBMV) with its hosts', we felt it appropriate to examine the possible induction of pathogenesis-related proteins (PRs) in our experimental bean and cowpea cultivars when exposed to a variety of stimuli.

In addition to single-lesion isolates of our first two chosen strains of SBMV [the bean (or type) strain and the cowpea strain], treatment of test plants with the vulgare strain of tobacco mosaic virus (TMV), to which plants are normally considered immune, and spraying with acetylsalicylic acid (1) were thought appropriate stimuli for study.

Plant cultivars chosen were *Phaseolus vulgaris* L. cvs Prince and Pinto, systemic and hypersensitive hosts, respectively, for the type strain of SBMV; and *Vigna sinensis* L. cvs Blackeye and Clay, the respective, corresponding hosts for the cowpea strain of SBMV.

Control plants of each cultivar were either left completely untouched, or were dusted with 300 grit carborundum and gently rubbed with a muslin pad soaked in 50 mM sodium phosphate buffer, pH 7.0, prior to washing in tap water.

After treatment, all experimental plants were grown for 8 days in a roof-top greenhouse with a supplemented 16-h photoperiod at ambient temperatures of 25 ± 5 °C. Batches of leaves were harvested, stored at -80 °C, ground while frozen and weighed. Protein was extracted by thorough grinding for 5-7 min in a chilled mortar and pestle, containing a dusting of 150 grit carborundum and 20 ml MacIlvaine buffer [84 mM citrate, 32 mM Na_2HPO_4, pH 2.8] containing 0.5M sucrose and 42mM (0.3% v/v) 2-mercaptoethanol. Fibre was removed by squeezing through muslin and the resulting extracts (30-35 ml each) were centrifuged for 30 min at 30 000 *g* at 4 °C. Each

supernatant was dialysed for 18-24 h at 4 °C against 2 l of 50 mM Tris-HCl, pH 8.0 containing 1mM EDTA and 3mM 2-mercaptoethanol, then against 2×2 l of double-distilled water, each for 12 h. Samples were then lyophilized.

As might be expected, fully-denaturing, discontinuous polyacrylamide slab gel electrophoresis on 17.5% (w/v) resolving gels (2) revealed a complex spectrum of proteins, more or less unique to each cultivar, with few notable differences between batches of plants exposed to different treatments. One possible candidate for a PR protein, with an apparent mol.wt of 26 000, appeared in the cowpea extracts (Fig. 1.).

Non-denaturing, discontinuous polyacrylamide slab gel electrophoresis on 10% (w/v) resolving gels (3) revealed a surprisingly uniform pattern of high mobility polypeptides (Rf range 0.50-1.00), among the variously-treated batches of each cultivar. In both bean cultivars, all experimental treatments, with the exception of the untreated controls revealed identical banding patterns, at comparable intensities (Fig. 2). Therefore, it would appear that physical or chemical damage alone can induce PRs in beans, possibly by permitting secondary bacterial or fungal infections to enter the plants. Even the untreated controls showed some lesser signs of putative PRs, perhaps reflecting the sensitivity of bean to the local heavily-polluted atmosphere around our greenhouse, which is surrounded by the extractor ventilators for nine-storeys of

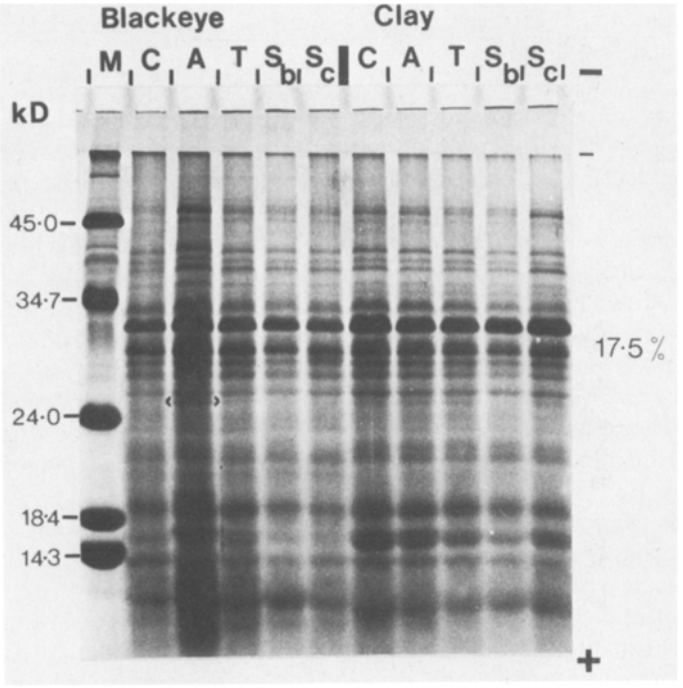

Fig. 1. Electrophoretic patterns in 17.5% polyacrylamide gel containing 0.1% SDS of pH 2.8-soluble proteins from cowpea cvs Blackeye and Clay. M: marker proteins; C: inoculated with buffer; A: treated aspirin; T: infected with TMV; S_b: infected with the bean (type) strain of SBMV; S_c: infected with the cowpea strain of SBMV. Note the appearance of a protein of MW 26 000 (< >).

laboratory fume-cupboards!

Cowpea extracts showed a more predictable correlation with batch treatment, but only in the relative intensities of the polypeptide of Rf 0.93-0.94. Again this was present in all plants; however aspirin, and to a lesser extent, TMV seemed to stimulate its presence in 'Blackeye' extracts. As with beans, 'Clay' cowpeas appeared more uniformly susceptible to abrasive-damage induction of PRs (Fig. 3).

These results are of interest in the light of previous reports on PR-protein synthesis

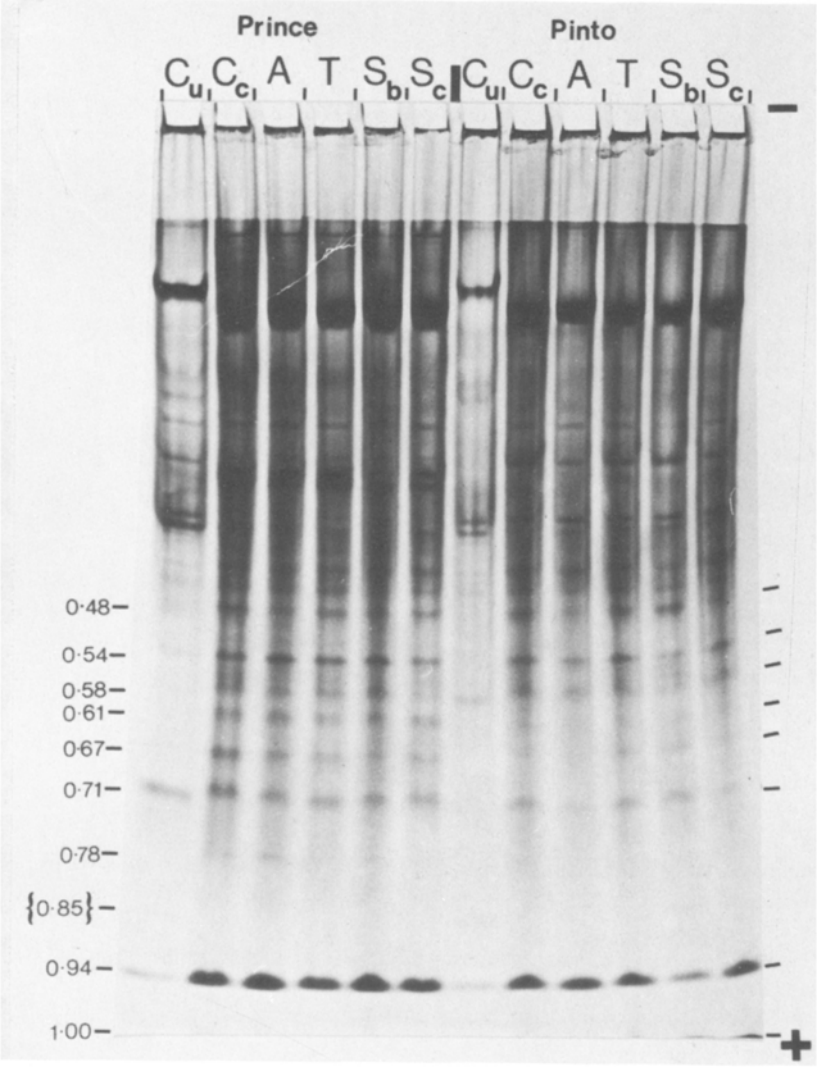

Fig. 2. Electrophoretic patterns in 10% polyacrylamide gel of pH 2.8-soluble proteins from bean cvs Prince and Pinto. Lettering as in Fig. 1, except C_u: untouched controls; C_c: carborundum-abraded controls.

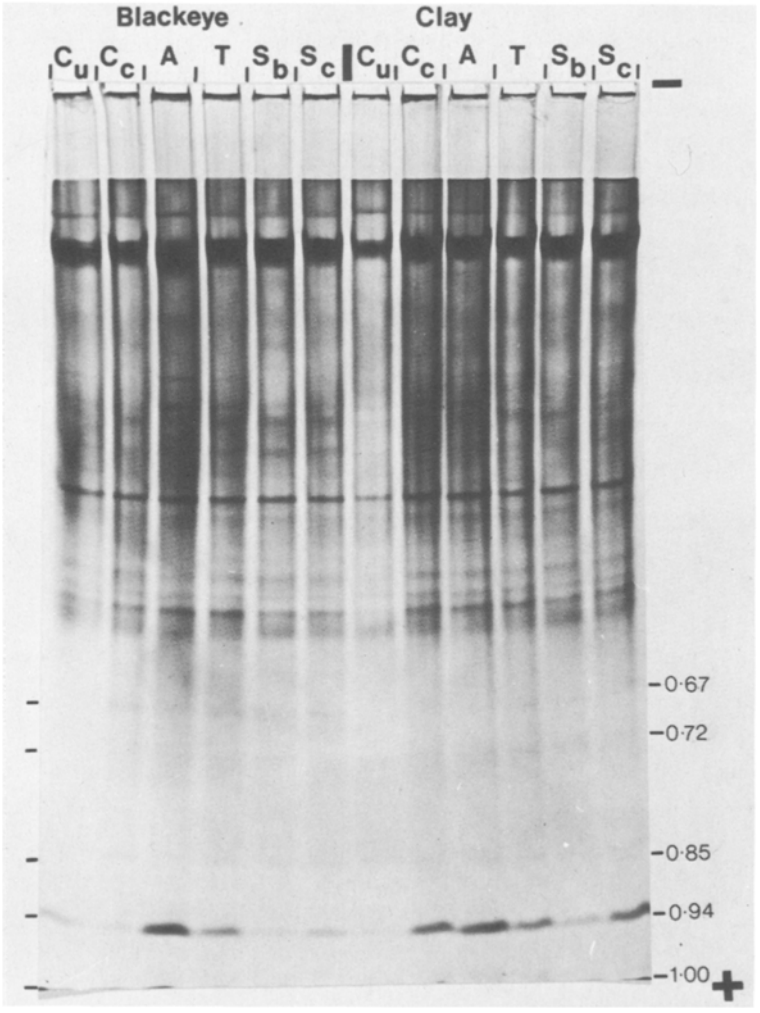

Fig. 3. Electrophoretic patterns in 10% polyacrylamide gel of pH 2.8-soluble proteins from cowpea cvs Blackeye and Clay. Lettering as in Figs 1 and 2.

in tobacco necrosis virus-infected cowpeas (4) and in beans infected with fungi or viruses, or treated with chemicals (5).

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Changes in protein patterns of bean leaves after bean rust infection or application of elicitor

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Changes in patterns of soluble proteins of bean leaves after infection with bean rust *(Uromyces phaseoli)* were studied by electrophoresis in polyacrylamide pore gradient gels (4%-25% acrylamide).

Four newly formed *acidic* proteins not present in healthy leaves were found in extracts of inoculated leaves of the resistant, hypersensitively reacting variety (017). Their mol. wts were estimated as 17 000 (P_1), 27 000 (P_2), 33 500 (P_3) and 34 000 (P_4) (in the absence of SDS). The most prominent protein, P_1, could be detected already 1 day and proteins P_2-P_4 2-3 days after inoculation; all increased in amount up to 8 days after inoculation. All new proteins were restricted to the hypersensitively reacting tissue. Changes were also detected for soluble *basic* proteins. Five bands which were faintly visible in healthy leaves of the resistant variety increased dramatically in intensity between the 4th and 8th day after inoculation with the fungus.

Four new proteins (identical or very similar in size to those found in the resistant bean variety) were induced in non-inoculated leaves of the susceptible variety Favorit by infiltration of a polyglucan which acts as an elicitor of phytoalexin synthesis (1). Inoculation with the fungus 2 h after infiltration with the elicitor resulted in a successful infection, whereas an infiltration 3 days *before* inoculation caused total protection, correlated with the presence of the four new proteins.

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Induction of pathogenesis-related (b) proteins in Phaseolus vulgaris upon fungal or viral infection or after chemical injury

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Primary leaves of French bean *(Phaseolus vulgaris* L.) cvs Brittle wax or Immuna react with necrotic lesions to infection with either *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. or alfalfa mosaic virus (AMV).

Electrophoresis in 10% polyacrylamide gels of the soluble leaf proteins present in extracts of leaves infected with *C. lindemuthianum* or AMV revealed three bands (p1, p2 and p3) which were not seen in extracts of healthy leaves. Foliar sprays with the growth regulator Aliette (Phosethyl-Al) at 2000 ppm (a.i) delayed the appearance of