Fundam. appl. Nematol., 1993, **16** (6), 549-556

Induction and detection of pathogenesis-related proteins in leaves and roots of potato plants infected with pathotypes of *Globodera pallida*

Siyavosh RAHIMI *+, Roland N. PERRY * and Denis J. WRIGHT +

* *Entomology & Nematology Department, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, UK and*
+ *Department of Biology, Imperial College at Silwood Park, Ascot, Berkshire, SL5 7PY, UK.*

Accepted for publication 17 April 1993.

Summary – The presence of pathogenesis-related (PR) proteins in leaves and roots of potato plants infected with *Globodera pallida* (Pa 1, Pa 2/3) or treated with chemicals has been examined using both denaturing and non-denaturing PAGE techniques. In the intercellular fluid from cv. Désirée infected with *G. pallida* Pa 1 and Pa 2/3 several new acidic proteins were resolved in non-denaturing 20 % homogenous gels, while 8-25 % gradient gels resolved only one major new band and a few minor ones. These new proteins have Rf values in the range 0.2 to 0.9. In cv. Maris Piper, six new proteins with Rf values in the same range were detected from plants infected with *G. pallida*. In denaturing SDS-PAGE new proteins were resolved in the intercellular fluid of infected plants of cv. P55/7; these new proteins have molecular weights in the range 14-45 kD. The cv. P55/7, which is considered to be resistant to *G. pallida* Pa 1 and susceptible to Pa 2/3, responds differently to attack by these pathotypes; the intensity of PR protein bands from intercellular fluid and leaf homogenate analyses was greater from plants invaded by juveniles of Pa 2/3 than from those attacked by Pa 1. Analysis of root extracts of plants infected with *G. pallida* revealed the presence of two higher molecular weight proteins with estimated molecular weights of 70 kD and 82 kD. All three cvs tested responded similarly to induction of PR proteins by salicylic acid and silver nitrate treatment, although the presence of PR proteins was more easily determined in cv. Maris Piper. These proteins had molecular weights in the range 14-45 kD.

Résumé – ***Induction et détection de protéines liées à la nocuité dans les feuilles et les racines de pomme de terre infestées par des pathotypes de*** **Globodera pallida** – La présence de protéines liées à la nocuité (PR) dans les feuilles et les racines de pieds de pomme de terre infestés par *Globodera pallida* (Pa 1, Pa 2/3) ou traités par des produits chimiques a été étudiée en utilisant les techniques PAGE dénaturante et non dénaturante. Dans le fluide intercellulaire provenant du cv. Désirée infesté par *G. pallida* Pa 1 et Pa 2/3, plusieurs protéines acides nouvelles sont mises en évidence dans 20 % des gels homogènes non dénaturants tandis que 8 à 25 % des gels à gradient ne mettent en évidence qu'une bande majeure nouvelle et quelques bandes mineures. Ces nouvelles protéines ont un Rf variant de 0,2 à 0,9. Chez le cv. Maris Piper, six nouvelles protéines ayant un Rf de même valeur ont été détectées dans des plants infestés par *G. pallida*. La technique SDS-PAGE dénaturante a mis en évidence de nouvelles protéines dans le fluide intercellulaire des plants infestés du cv. P55/7; ces nouvelles protéines ont un poids moléculaire variant de 14 à 45 kD. Le cv. P55/7, considéré comme résistant à *G. pallida* Pa 1 et sensible à Ps 2/3, réagit différemment aux attaques de ces pathotypes; l'intensité des bandes caractérisant les protéines PR provenant du fluide intercellulaire et d'homogénats de feuilles est plus élevée dans le cas d'infestation par des juvéniles de Pa 2/3 que dans celui de juvéniles de Pa 1. L'analyse d'extraits de racines de plants infestés par *G. pallida* révèle la présence de deux protéines de poids moléculaire élevé, estimé à 70 et 82 kD. Les trois cultivars testés répondent de la même manière à l'induction de protéines PR par un traitement à l'acide salicylique et au nitrate d'argent bien que la présence de ces protéines soit plus aisément détectée chez le cv. Maris Piper. Ces protéines ont un poids moléculaire variant de 14 à 45 kD.

Key-words : Nematodes, *Globodera pallida,* pathogenesis related proteins, invasion.

Pathogenesis-related (PR) proteins form a group of proteins induced in plants subjected to pathological and other stress related situations. They were first discovered independently by Gianinazzi *et al.* (1970) and van Loon and van Kammen (1970) in tobacco plants infected with tobacco mosaic virus (TMV). PR proteins have since been shown to be induced by other pathogens, such as bacteria (Ahl *et al.,* 1981) and fungi (Gianinazzi *et al.,* 1980), in a wide range of host plants (White *et al.,* 1987). Some of these polypeptides have relatively low molecular weights, accumulate extracellularly in infected plant tissue, exhibit high resistance to proteolytic enzymes and often possess extreme isoelectric points (Kombrink *et al.,* 1987). Several chemicals, such as salicylic acid (White, 1979), ethephon (van Loon, 1977), L-methionine (Asselin *et al.,* 1985), manganese chloride (White *et al.,* 1986) and ionic silver (Conejero & Granell, 1986), have also been shown to induce production of PR proteins; the effects of these chemicals have since been examined extensively

(Ohashi & Matsuoka, 1985, 1987; Asselin *et al.,* 1985; Ohashi *et al.,* 1986; Hoof van Huijduijnen *et al.,* 1986; Granell *et al.,* 1987; Vera & Conejero, 1989, 1990). The role and function of PR proteins in plants is not clearly understood. They are produced in large quantities in hypersensitive and resistant reactions. In tobacco infected with tobacco mosaic virus (TMV), although the presence of PR proteins does not lead to localization of TMV around lesions, it leads to induced resistance (Dumas & Gianinazzi, 1986). However, Sherwood (1985) concludes that PR proteins are a result of pathogen-induced necrosis and not significantly involved in the mechanism(s) of viral induced resistance.

Several classes of PR proteins have been identified in different species and/or families of plants. In tobacco plants, more than ten PR proteins were detected by van Loon (1982) and Jamet *et al.* (1985). An additional ten PR proteins were detected by Hogue and Asselin (1986) in tobacco. Nine acidic and six basic proteins and several minor ones were found in fifteen potato cultivars tested by Parent and Asselin (1987). In tomato plants, several classes of these proteins have also been identified. Some of these proteins exhibit serological relationships with each other while others are unrelated.

The induction of PR proteins by plant parasitic nematodes has been reported in only one instance. Hammond-Kosack *et al.* (1989) used denaturing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to study systemic accumulation of novel proteins in the apoplast of the leaves of potato plants following root invasion by the potato cyst nematode *Globodera rostochiensis,* pathotypes Ro1, Ro2, Ro3 and Ro4. In the present work we have studied the presence of PR proteins in leaves and roots of potato plants infected with *G. pallida* (Pa 1, Pa 2/3) using both denaturing and non-denaturing PAGE techniques. We have also tested the ability of several chemicals to induce production of PR-proteins.

Materials and methods

Potato tubers

Healthy tubers of three cultivars of potato were selected. Maris Piper, carrying the H1 gene which confers resistance to *G. rostochiensis* Ro1 but not to Ro5 or *G. pallida* pathotypes; Désirée, which is susceptible to all pathotypes of both species of potato cyst nematodes, and cv. P55/7 with the H2 gene from *Solanum multidissectum* which confers resistance to Pa 1 only (Dunnett, 1961). Potato tuber pieces with a single sprout were cut into small cubes (approximately 27 cm^3), left to heal for half an hour and were then sown singly in 12 cm plastic pots in 1:1 mixture of autoclaved sand and loam and kept in a glasshouse at 18 °C.

Nematode treatment

Cysts of *G. pallida,* Pa 1 and Pa 2/3, were from stock cultures established on differential potato cultivars at Rothamsted Experimental Station. Cysts and hatched juveniles of both pathotypes were used as inocula in separate trials. In tests using cysts as the inoculum, dry cysts were incorporated into the soil at the time of planting potato tuber pieces; the majority of juveniles from these cysts hatch within 2 to 3 weeks. In tests using hatched second stage juveniles as the inoculum, cysts were first soaked in glass distilled water (GDW) for a week and then placed in potato root diffusate (PRD). PRD was obtained by the method of Fenwick (1949) from 10 week-old potato plants (cv. Désirée) grown in sterile loam pot cultures in a glasshouse; PRD was diluted 1 in 4 by volume with GDW before use. Potato plants were inoculated 2 weeks after planting. A suspension of freshly hatched (less than 3 days) juveniles was poured into three disposable pipette tips (200-1000 µl) per pot, inserted into the soil near to the roots. Several treatment rates of cysts (20 to 50 cysts per pot) and juveniles (2000 to 5000 juveniles per pot) were used to test the relationship between level of infection and amount of PR proteins produced. There were five replicates for each treatment.

Chemical treatments

Salicylic acid ($HO{\cdot}C_6H_4COOH$) (1, 5, 10 mM), L-methionine ($C_5H_{11}NO_2S$) (10 mM), manganese chloride ($MnCl_2{\cdot}4H_2O$) (10 mM) and silver nitrate ($AgNO_3$) (1, 5, 10 mM) were neutralized to pH 7.0 with 1 N sodium hydroxide (NaOH) and were then used to spray the leaves of 2 to 3 week-old potato plants with five replicates per treatment. Chemicals were washed off with GDW spray 2 h after treatment and leaves were tested for the presence of PR proteins 4 and 7 days after treatment. Control plants were treated in exactly the same manner except the treatment spray was GDW.

Estimation of juvenile invasion

At each protein extraction interval (see below), a small (1 g) amount of roots was fixed in 5 ml of FAA fixative (20 ml 95 % ethanol; 6 ml 40 % formaldehyde; 1 ml glacial acetic acid; 40 ml GDW) and was stained by dipping in boiling acid fuchsin solution (0.005 % acid fuchsin in 1:1:1 lactic acid : glycerol : GDW) for 1.5 min. The stained roots were rinsed and then macerated in a blender for 30 s. A 200 ml suspension with GDW was made and 2 × 20 ml of this was pipetted into counting dishes and the number of nematodes per g of root which had invaded in each treatment was estimated.

Extraction of proteins

Proteins were analysed from three areas of plant tissue : the intercellular fluid of young leaves, from homogenates of leaves after intercellular fluid had been extracted and from root extracts. Proteins were extracted from 3 to 8 week-old plants.

The intercellular fluid of the leaves was extracted according to the method of Dewit and Spikman (1982) with some modifications. A known weight (10 g) of leaves was vacuum infiltrated under cool conditions (5 - 10 °C) for 30 min in a 250 ml beaker filled with GDW. The leaves were then blotted dry and centrifuged for 15 min at 8000 rpm at 4 °C. The supernatant was pipetted into Eppendorf tubes and stored frozen at – 20 °C before use.

To obtain extracts from leaves after the intercellular fluid had been removed, the leaves were homogenised in a mortar and pestle with 1 ml of cold citrate-phosphate buffer pH 3.0. The extracts were passed through polyester voile and then filtered through a Whatman No. 1 filter paper before being centrifuged at 15 000 rpm for 20 min at 4 °C. The supernatant was stored as before.

To extract protein from roots, 5-10 g of the roots were washed in several changes of GDW, blotted dry and homogenised in a mortar and pestle. The extract was passed through polyester voile and centrifuged and stored as for leaf extracts. Prior to analysis, extracts were centrifuged at 35 000 rpm for 30 min at 4 °C.

Polyacrylamide gel electrophoresis

Both denaturing and non-denaturing PAGE analysis were used and a minimum of 3 gels was run for each extract. For denaturing gels, extracts were boiled for 5 min in buffer (125 mM Tris base pH adjusted to 6.8 with 3 M HCl containing 0.4 % (w/v) SDS, 10 % (w/v) glycerol, 4 % (v/v) 2-mercaptoethanol and 0.02 % (w/v) bromphenol blue). Protein quantity was determined using the Coomassie spot test (Harlow & Lane, 1988) with bovine serum albumin as the standard; 4 mg ml^{-1} protein was used for each track. Samples from intercellular fluid, leaf homogenates and root tissue were run on slab gels using 3.9 % (w/v) acrylamide stacking gels and 12 % (w/v) separating gels (Laemmli, 1970) and either an ATTO Mini Dual Slab AE6450 or an ATTO Middy Slab AE6210 (Genetic Research Instrumentation Ltd, Dunmow, Essex, U.K.). Marker tracks were run routinely for molecular weight calculations. The gels were stained with Coomassie blue R-250 and then silver stained following standard procedures (BioRad).

In addition, the Phast system (Pharmacia) was used to detect new acidic proteins from intercellular fluid using non-denaturing 20 % homogeneous and 8-25 % gradient gels which were stained automatically with silver nitrate. Basic proteins were run on acidic gels (Hames, 1990) and stained with Coomassie blue R-250.

Results

To optimise the chances of detecting new proteins, extractions of proteins from leaves and roots were done at weekly intervals from the first week after nematode inoculation to the sixth week.

Induction of *PR* proteins by *G. pallida*

In the intercellular fluid from leaves of cv. Désorée infected with *G. pallida* Pa 1 and Pa 2/3 as many as ten acidic proteins, with Rf values of 0.2 to 0.9, were new or showed increased intensity in non-denaturing 20 % homogeneous gels (Fig. 1 A), while 8-25 % gradient gels resolved only one major new band and two minor ones with Rf values of approximately 0.6 (Fig. 1 B). In cv. Maris Piper, six new proteins with Rf values of 0.5 to 0.9 and molecular weights of 14-45 kD were detected from plants infected with *G. pallida* (Fig. 1 C). As well as production of new proteins, the results indicate that invasion by *G. pallida* also results in a reduction of proteins : a protein with an Rf value of approximately 0.75 is present in the uninfected controls (arrowed in track III, Fig. 1 A) but is absent or markedly reduced in infected cv. Désirée. This could be a cultivar specific protein because this protein was not detectable in cv. Maris Piper (Fig. 1 C, track V). One major band with an Rf value of 0.6 (arrowed in track V, Fig. 1 C) was present in both uninfected and infected cv. Maris Piper but, although absent in uninfected cv. Désirée, the protein was induced in cv. Désirée infected with both pathotypes of *G. pallida*. However, until extracts of different cultivars are run on the same gel it is not possible to be definite about cultivar specific proteins.

In 12 % homogeneous SDS-PAGE new proteins were resolved and some of the pre-existing proteins increased in intensity in the intercellular fluid of infected plants of cv. P55/7 (Fig. 2 A, track II); these new proteins have molecular weights in the range 14-45 kD. The cv. P55/7, which is considered to be resistant to *G. pallida* Pa 1 and susceptible to Pa 2/3, responds differently to attack by these pathotypes; the PR protein bands obtained from intercellular fluid were consistently more intense from cv. P55/7 invaded by juveniles of Pa 2/3 than when attacked by Pa 1 (Fig. 2 A).

Denaturing PAGE analysis of leaf homogenates of infected plants of all three cvs showed that several of the pre-existing bands had increased markedly in intensity (Fig. 2 B shows the response of cv. P55/7); however, it was difficult to determine with certainty the presence of new proteins. These pre-existing bands, with molecular weights in the range 24 to 66 kD, were more intense in leaf homogenates from cv. P55/7 infected with Pa 2/3 than in leaf homogenates from plants inoculated with the same numbers of Pa 1; however, increase in the inoculum levels of Pa 1 resulted in increased intensity of these protein bands (Fig. 2 B, track IV).

Analysis of root extracts of cv. P55/7 inoculated with Pa 1, revealed the presence of two high molecular weight proteins (Fig. 2 C, track I). By running different molecular markers we have been able to estimate the molecular weight of these two proteins as approximately 70 kD and 82 kD (data not shown). Preliminary work

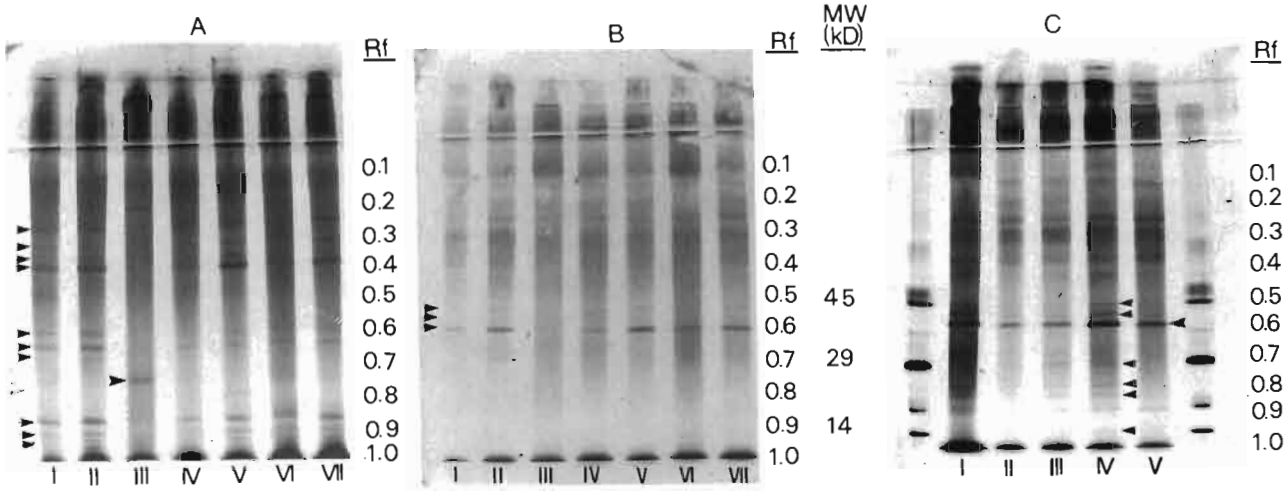

Fig. 1. A, B : Non-denaturing PAGE of acidic proteins from intercellular fluid of 4 week old potato plants, cv. Désirée, inoculated with cysts of *G. pallida,* Pa 1 and Pa 2/3. Track III is from uninfected control plants. Tracks I, IV, VI are from plants inoculated with 20, 40 and 60 cysts of Pa 1, respectively. Tracks II, V, VII are from plants inoculated with 20, 40 and 60 cysts of Pa 2/3, respectively; C : Non-denaturing PAGE of acidic proteins from intercellular fluid of 4 week old potato plants, cv. Maris Piper, infected with second stage juveniles of *G. pallida,* Pa 1. Track V is from uninfected control plants. Tracks I to IV are from plants inoculated with 1000, 2000, 3000, 4000 second stage juveniles, respectively.
(Gel A is 20 % homogeneous and B and C are 8-25 % gradient. The gels were stained with silver nitrate. Proteins which are new or show increased intensity are arrowed. Molecular weight markers are shown on either side of Fig. 1 C.)

with antibodies shows that these two proteins are not nematode proteins (Rahimi & Perry, unpubl.).

INDUCTION OF *PR* PROTEINS BY CHEMICAL TREATMENT

Analysis of intercellular fluid from plants treated with chemicals, demonstrated that salicylic acid and, especially, silver nitrate induced production of PR proteins. Salicyclic acid at 5 mM was the optimum concentration for induction of new proteins; at concentrations greater than this severe necrosis of the leaves occurred and below 5 mM the chemical was not very effective (Fig. 3 A). Silver nitrate at 10 mM concentration induced PR proteins or caused increased intensity in some of the existing proteins (Fig. 3 B, tracks IV) without causing severe necrosis. All three cvs tested responded similarly to chemical induction of PR proteins although the presence of PR proteins was more easily determined in cv. Maris Piper (Fig. 3 A, tracks I-IV). These proteins had molecular weights in the range 14-45 kD.

Although the resolution is poor using acidic gels, some basic protein bands appear or become more intense in extracts from infected and chemically treated plants compared with the controls (Fig. 3 C, D). Further work on basic proteins is required to confirm these differences.

Discussion

Expression of the genes coding for PR proteins does not normally occur in healthy developing plants but is triggered in response to infection by a wide range of pathogens (Antoniw & White, 1987) and by abiotic stress conditions such as chemical treatment (Sehgal & Mohamed, 1990). Hammond-Kosack *et al.* (1989) used denaturing SDS-PAGE to study systemic accumulation of novel proteins in the apoplast of the leaves of potato plants following root invasion by *G. rostochiensis;* the proteins were not cultivar or pathotype specific. They attempted to synchronise invasion by nematodes which resulted in a large infection over a short, concentrated time span. As this does not relate to natural invasion, especially with *G. pallida* which hatches over a much longer period than *G. rostochiensis* (Robinson *et al.*, 1987), we used cysts as the inoculum to monitor the plant response in relation to PR protein production under more natural invasion regimes; hatched juveniles were also used in a comparison to represent more concentrated invasion. Sampling over 1 to 6 weeks after infection enabled us to examine if PR proteins were all produced at the same time after invasion. There was no difference in rate of production of PR proteins as all appeared at the same time with an optimum period for resolution of PR proteins of 2-3 weeks after inoculation with J2s and 4-5 weeks with cysts. There was also no difference in the number of PR proteins produced with increase in J2 numbers and there was no difference in PR proteins whether J2 or cysts were used as inoculum.

The PR proteins detected from infected potato leaves and roots, using denaturing and non-denaturing PAGE systems, show common features with PR proteins dis-

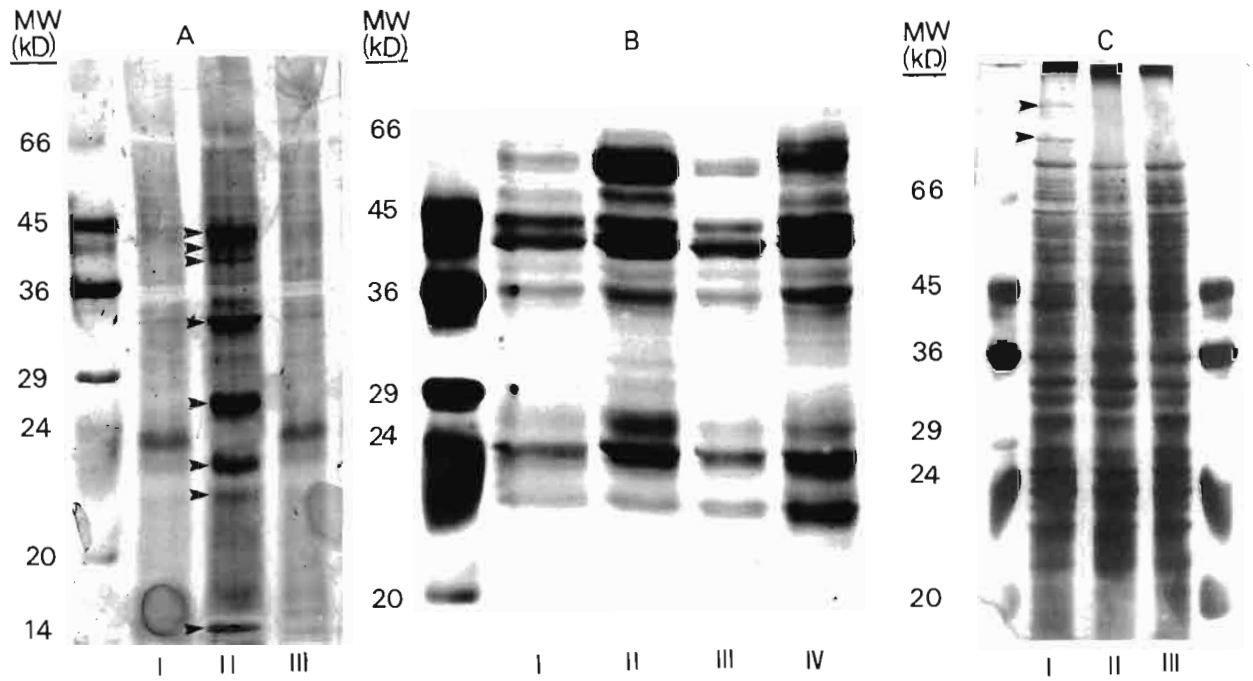

Fig. 2. A : 12 % SDS-PAGE of proteins from intercellular fluid of 4 week old potato plants, cv. P55/7, infected with *G. pallida*. Track III is from the uninfected control plants. Tracks I and II are from plants inoculated with 30 cysts of Pa 1 and Pa 2/3, respectively. Proteins which are new or show increased intensity are arrowed. Molecular weight markers are on the left hand side of the gel; B : 12 % SDS-PAGE of leaf homogenates from 4 week old potato plants cv. P55/7 infected with second stage juveniles of *G. pallida*. Track III is from the uninfected control plants. Tracks I and IV are from plants inoculated with 3000 and 5000 juveniles of Pa 1, respectively, and track II is from plants inoculated with 3000 juveniles of Pa 2/3. Molecular weight markers are on the left hand side of the gel; C : 12 % SDS-PAGE of root extracts of 5 weeks old potato plants, cv. P55/7 inoculated with 30 cysts of Pa 1 (track I) and 30 cysts of Pa 2/3 (track II). Track III is from the uninfected control plants. Note the presence of two extra bands (arrowed) in track I with approximate molecular weights of 70 kD and 82 kD. Molecular weight markers were run on both sides of the gel. (The gels were stained with Coomassie blue R-250 and then with silver nitrate.)

covered in other plant-pathogen interactions. For example, they could be extracted at low pH and had low molecular weights. The PR proteins detected in this work using SDS-PAGE had molecular weights in the range 14-45 kD and are similar in size to those detected previously (van Loon *et al.*, 1987; van Loon & Gerritsen, 1989; Fischer, *et al.*, 1989; Kauffmann, *et al.*, 1990). The SDS-PAGE of root extracts revealed the presence of two higher molecular weight proteins. These proteins have molecular weights similar to those detected in intercellular fluid of tomato infected with *Phytophthora infestans* (Fischer *et al.*, 1989). A comparison between our results and those of Hammond-Kosack *et al.* (1989) shows that there are several PR proteins which may be similar; PR proteins with molecular weights 14 kD, 29 kD, 33 kD and 45 kD appear to be present in both studies.

There has been a great deal of speculation about the function of PR proteins in infected plants. PR proteins may be involved in induced resistance either in localized or in systemic form (Antoniw & White, 1987). They may play a role in defence across the cell wall of plants as these proteins are found abundantly in the intercellular spaces between the cells (Legrand *et al.*, 1987). In our work, expression of PR proteins appears to be part of a generalised response in that they were produced in the infection process irrespective of cultivar or nematode pathotype; they were also produced in response to chemical treatment. There are currently no commercially available potato cultivars with complete resistance to *G. pallida*. The experimental cv. P55/7 was included in this work because it is resistant to Pa 1 but susceptible to Pa 2/3 and may have provided a useful experimental model to examine pathogen-specific responses which may relate to resistance. It is interesting to note that cv. P55/7 responds differently to attack by pathotypes of *G. pallida;* the analysis of intercellular fluid and leaf homogenates revealed greater amounts of new proteins in the plants infected with Pa 2/3 than from plants infected with Pa 1 (Fig. 2 A, B). This is contrary to what would be expected from the purported role of PR proteins in resistance mechanisms. Future work will centre on the use of antibodies to identify and localize some of these new proteins.

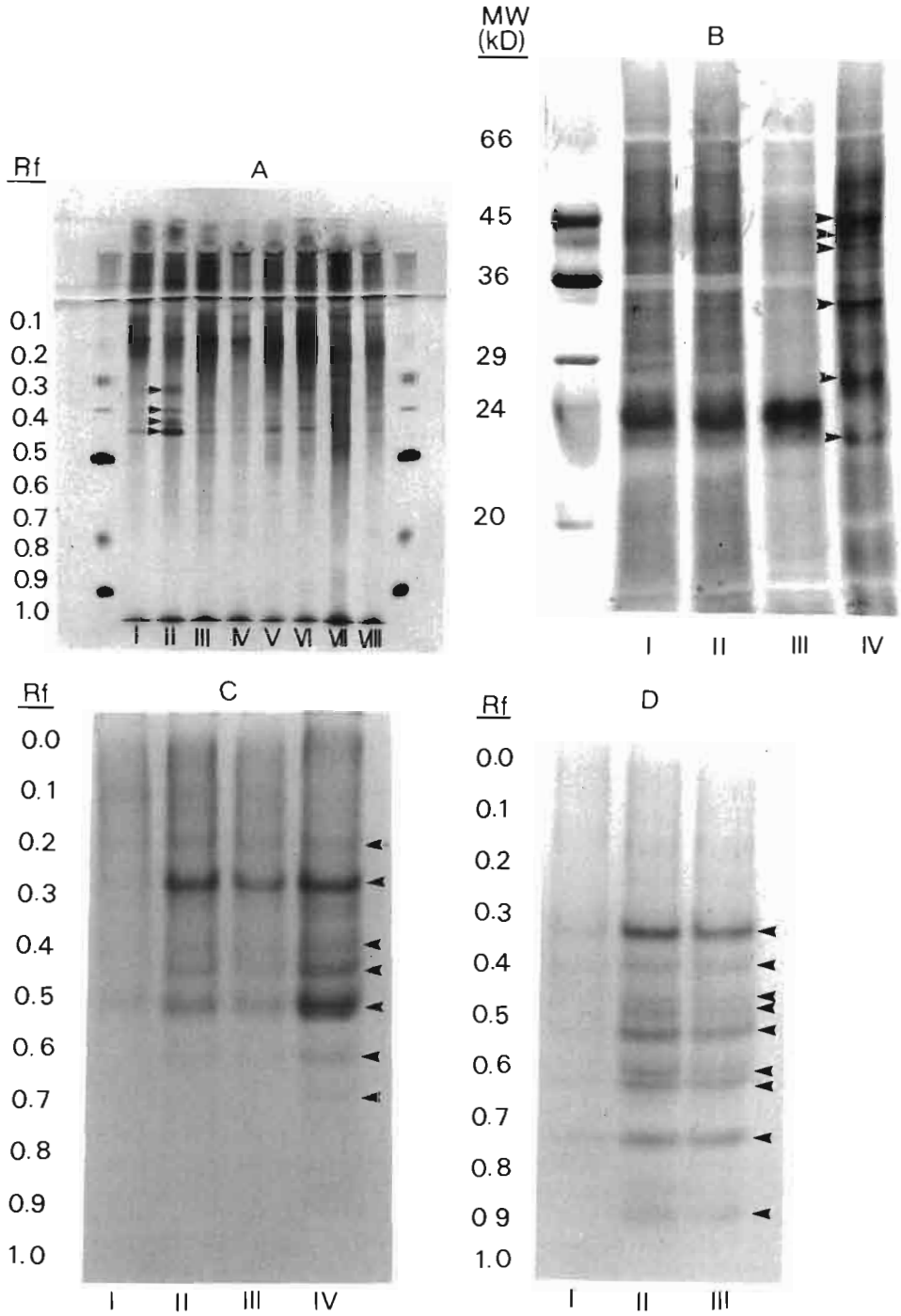

Fig. 3. A : 20 % non denaturing PAGE of intercellular fluid showing the effect of treatment with salicylic acid on potato plants, cvs Maris Piper (tracks I-IV) and Désirée (V-VIII). The chemical was sprayed on 3 week old plants and the intercellular fluid was extracted 1 week later. Tracks IV & VIII are from the untreated control plants. Tracks I & V are from plants sprayed with 1 mM salicylic acid, tracks II & VI are from the 5 mM salicylic acid treatment and tracks III & VII are from the 10 mM salicylic acid treatment; B : 12 % SDS-PAGE of intercellular fluid from potato plants, cv. P55/7, treated with chemicals. The chemicals were sprayed on 5 week-old plants and the intercellular fluid was extracted 1 week later. Track I is from plants treated with 10 mM manganese chloride, track II is from the 10 mM L-methionine treatment, track III is from the untreated control plants and track IV is 10 mM silver nitrate; C : 7 % native basic PAGE of proteins from leaf homogenates of 7 week-old potato plants, cv. P55/7. Track I is from the uninfected control plants, track II is *G. pallida* Pa 1 (50 cysts inoculum), track III is Pa 2/3 (50 cysts inoculum) and track IV is 10 mM silver nitrate treatment; D : 7 % native basic PAGE of proteins from root extracts of 7 week-old potato plants, cv. P55/7. Track I is from the unifected control plants. Track II is Pa 1 (50 cysts inoculum) and track III is Pa 2/3 (50 cysts inoculum). (Gel A was stained with silver nitrate and gel B was stained with Coomassie blue R-250 and then with silver nitrate. Gels C and D were stained with Coomassie blue R-250. Proteins which are new or show increased intensity are arrowed.)

Acknowledgements

We are grateful to Mr. R. F. White and Dr. J. F. Antoniw, Rothamsted Experimental Station, for their advice and help with this work. We thank Dr. Ingrid Clayden, Scottish Agricultural Science Agency, for providing potato cv. P55/7.

References

AHL, P., BENJAMA, A., SAMSON, R. & GIANINAZZI, S. (1981). Induction chez le tabac par *Pseudomonas syringae* de nouvelles protéines (protéines " b ") associées au développement d'une résistance non spécifique à une deuxième infection. *Phytopath. Z.,* 102 : 201-212.

ANTONIW, J. F. & WHITE, R. F. (1987). The role of pathogenesis-related proteins. *Ciba Found. Symp.,* 133 : 57-71.

ASSELIN, A., GRENIER, J. & COTE, F. (1985). Light-influenced extracellular accumulation of 6 (Pathogenesis-related) proteins in *Nicotiana* green tissue induced by various chemicals or prolonged floating on water. *Can. J. Botany,* 63 : 1276-1283.

CONEJERO, V. & GRANELL, A. (1986). Stimulation of a viroid-like syndrome and the impairment of viroid infection in *Gynura aurantiaca* plants by treatment with silver ions. *Physiol. molec. Pl. Pathol.,* 29 : 317-323.

DEWIT, P. J. G. M. & SPIKMAN, G. (1982). Evidence for the occurrence of race and cultivar-specific elicitors of necrosis in intercellular fluids of compatible interactions of *Cladosporium fulvum* and tomato. *Physiol. Pl. Pathol.,* 21 : 1-11.

DUMAS, E. & GIANINAZZI, S. (1986). Pathogenesis-related (b) proteins do not play a central role in TMV localization in *Nicotiana rustica. Physiol. molec. Pl. Pathol.,* 28 : 243-250.

DUNNETT, J. M. (1961). Inheritance of resistance to potato root eelworm in a breeding line stemming from *Solanum multidissectum* Hawkes. *Scott. Pl. Breed. Stn Rep.* 1961 : 39-46.

FENWICK, D. W. (1949). Investigations on the emergence of the larvae from cysts of the potato-root eelworm *Heterodera rostochiensis.* I. Technique and variability. *J. Helminth.,* 23 : 157-170.

FISCHER, W., CHRIST, U., BAUMGARTNER, M. & ERISMANN, K. H. (1989). Pathogenesis-related proteins of tomato : II- Biochemical and immunological characterization. *Physiol. molec. Pl. Pathol.,* 35 : 67-83.

GIANINAZZI, S., MARTIN, C. & VALLÉE, J. C. (1970). Hypersensibilité aux virus, température et protéines solubles chez le *Nicotiana tabacum* Xanthi-nc. Apparition de nouvelles macro-molécules lors de la répression de la synthèse virale. *C. r. hebd. Acad. Sci., Paris, Sér. D,* 270 : 2383-2386.

GIANINAZZI, S., AHL, A., CORNU, A. & SCALLA, R. (1980). First report of host b-protein appearance in response to a fungal infection in tobacco. *Physiol. Pl. Pathol.,* 16 : 337-342.

GRANELL, A., BELLS, J. M. & CONEJERO, V. (1987). Induction of pathogenesis-related proteins in tomato by citrus exocortis viroid, silver ion and ethephon. *Physiol. molec. Pl. Pathol.,* 31 : 83-90.

HAMES, B. D. (1990). Gel electrophoresis of proteins. *In :* Hames, B. D. & Rickwood, D. (Eds). *One dimentional polyacrylamide gel electrophoresis.* Oxford & New York, IRL Press : 1-147.

HAMMOND-KOSACK, K. E., ATKINSON, H. J. & BOWLES, D. J. (1989). Systemic accumulation of novel proteins in the apoplast of the leaves of potato plants following root invasion by the cyst nematode *G. rostochiensis. Physiol. molec. Pl. Pathol.,* 35 : 495-506.

HARLOW, E. & LANE, D. (1988). *Antibodies - a laboratory manual.* Cold Spring Harbor Laboratory, 726 p.

HOGUE, R. & ASSELIN, A. (1986). Detection of 10 additional pathogenesis-related (b) proteins in intercellular fluid extracts from stressed " Xanthi "-nc tobacco leaf tisue. *Canad. J. Botany,* 65 : 476-481.

HOOFT VAN HUIJDUIJNEN, R. A. M., ALBIAS, S. W., DERIJK, R. H. & BOL, J. F. (1986). Induction by salicylic acid of pathogenesis-related proteins and resistance to Alfalfa mosaic virus infection in various plant species. *J. gen. Virol.,* 67 : 2135-2143.

JAMET, E., KOPP, M. & FRITIG, B. (1985). The pathogenesis-related proteins of tobacco : their labelling from [^{14}C] amino acids in leaves reacting hypersensitively to infection by tobacco mosaic virus. *Physiol. Pl. Pathol.,* 27 : 29-41.

KAUFFMANN, S., LEGRAND, M. & FRITIG, B. (1990). Isolation and characterisation of six pathogenesis-related (PR) protein of Samsun NN tobacco. *Pl. Molec. Biol.,* 14 : 381-390.

KOMBRINK, E., SCHRÖDER, M. & HAHLBROCK, K. (1987). Several pathogenesis-related proteins in potato are 1,3 β-glucanases and chitinases. *Proc. natn. Acad. Sci. USA,* 85 : 782-786.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature,* 227 : 680-685.

LEGRAND, M., KAUFFMANN, S., GEOFFROY, P. & FRITIG, B. (1987). Biological function of pathogenesis-related proteins : four tobacco pathogenesis-related proteins are chitinases. *Proc. natn. Acad. Sci. USA,* 84 : 6759-6754.

OHASHI, Y. & MATSUOKA, M. (1985). Precise mode of induction of pathogenesis-related proteins by chemical treatment in tobacco. *Proc. Japan Acad., Ser. B,* 61 : 391-394.

OHASHI, Y. & MATSUOKA, M. (1987). Induction and secretion of pathogenesis-related proteins by salicylate or plant hormones in tobacco suspension cultures. *Pl. Cell Physiol.,* 28 : 573-580.

OHASHI, Y., SHIMOMURA, T. & MATSUOKA, M. (1986). Acquisition of resistance to TMV coincident with induction of pathogenesis-related proteins by TMV infection and chemical treatment in tobacco leaves. *Ann. phytopath. Soc. Japan,* 52 : 626-635.

PARENT, J. G. & ASSELIN, A. (1987). Acidic and basic extracellular pathogenesis-related leaf proteins from fifteen potato cultivars. *Phytopathology,* 77 : 1122-1125.

ROBINSON, M. P., ATKINSON, H. J. & PERRY, R. N. (1987). The influence of temperature on the hatching, activity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida. Revue Nématol.,* 10 : 349-354.

SEHGAL, O. P. & MOHAMED, F. (1990). Pathogenesis-related proteins. *In :* Mandahar, C.L. (Ed.). *Plant viruses.* Boca Ratoon & Boston, CRC Press : 65-83.

SHERWOOD, J. L. (1985). The association of " pathogenesis-related " proteins with viral induced necrosis in *Nicotiana sylvestris. Phytopath. Z.,* 112 : 48-55.

VAN LOON, L. C. (1977). Induction by 2-Chloroethylphosphonic acid of viral-like lesions, associated proteins, and systemic resistance in tobacco. *Virology;* 80 : 417-420.

VAN LOON, L. C. (1982). Regulation of changes in proteins and enzymes associated with active defence against virus infection. *In :* Wood, R.K.S. (Ed.). *Active defence mechanisms in plants.* New York & London, Plenum Press : 247-273.

VAN LOON, L. C. & GERRITSEN, Y. A. M. (1989). Localization of pathogenesis-related proteins in infected and non-infected leaves of Samsun NN tobacco during the hypersensitive reaction to tobacco mosaic virus. *Pl. Sci.,* 63 : 131-140.

VAN LOON, L. C. & VAN KAMMEN, A. (1970). Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var " Samsun " and " Samsun NN ". Changes in protein constitution after infection with TMV. *Virology,* 40 : 199-201.

VAN LOON, L. C. & GERRITSEN, Y. A. M. & RITTER, C. E. (1987). Identification, purification and characterization of pathogenesis-related proteins from virus infected Samsun NN tobacco leaves. *Pl. molec. Biol.,* 9 : 593-609.

VERA, P & CONEJERO, V. (1989). The induction and accumulation of the pathogenesis related P 69 proteinase in tomato during citrus exocortis viroid infection and in response to chemical treatments. *Physiol. molec. Pl. Pathol.,* 34 : 324-334.

VERA, P. & CONEJERO, V. (1990). Effect of ethephon on protein degradation and the accumulation of " pathogenesis-related " (PR) proteins in tomato leaf discs. *Pl. Physiol.,* 92 : 227-233.

WHITE, R. F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology,* 99 : 410-42.

WHITE, R. F., DUMAS, E., SHAW, P. & ANTONIW, J. F. (1986). The chemical induction of PR (b) proteins and resistance to TMV infection in tobacco. *Antiviral Res.,* 6 : 177-185.

WHITE, R. F., RYBICKI, E. P., WECHMAR, M. B., DEKKER, J. J. & ANTONIW, J. F. (1987). Detection of PR 1 type protein in Amaranthaceae, Chenopodiaceae, Graminae and Solanaceae by immunoelectroblotting. *J. gen. Virol.,* 68 : 204-2043.