EXTRACELLULAR ENZYMES OF *CHONDROSTEREUM PURPUREUM*, CAUSAL FUNGUS OF SILVERLEAF DISEASE

R.M. SIMPSON, R. VAN HEKEZEN, F. VAN LUNE, D. BREWSTER,
J.T. CHRISTELLER and A.G. SPIERS

*Horticulture and Food Research Institute of New Zealand, Private Bag 11030,
Palmerston North, New Zealand*

Corresponding author: rsimpson@hortresearch.co.nz

ABSTRACT

Chondrostereum purpureum, the casual fungus of silverleaf disease, was grown on solid media supplemented with a range of oligosaccharide carbon sources. Extracellular extracts were analysed for a range of wood-degrading enzymes. The results indicate that *C. purpureum* is a highly specialised plant pathogen which does not produce lignin degrading enzymes. Cellulose substrates produced slow growth of the fungus, but induced large amounts of enzymes able to degrade celluloses, xyloglucans and pectins. Growth on Link pectin and pectin was rapid, with the induction of enzymes able to degrade pectins and cellulases. The growth data suggests that the cellulases especially, but also pectinases, play a key role in xylem invasion rather than in nutrition. The fungus also has the capacity to utilise protein and starch. The results are discussed in relation to the local and systemic effects of the fungus.

Keywords: *Chondrostereum purpureum*, silverleaf disease, pectinases, cellulases, enzyme induction.

INTRODUCTION

The silverleaf fungus (*Chondrostereum purpureum*) is a basidiomycete plant pathogen which adventitiously invades a wide range of plant species through wounds (Spiers & Hopcroft 1987). The disease is characterised by fungal growth local to the infection site through the xylem tissues of the wood (Pearce et al. 1994) and by a systematic silvering of leaves and softening of fruit (Spiers et al. 1987). The loss of yield due to reduced photosynthetic capacity and the direct effects on fruit quality can be economically significant in pip and summerfruit crops. The spread of the fungus and the loss of photosynthetic capability eventually leads to tree death and formation of fungal fruit bodies.

Extracellular enzyme activity is not only essential for fungal growth through degradation of plant cells to provide nutrients, but has also been implicated in causation of the systemic effects. Recently the enzyme endopolygalacturonase, isolated from media on which the fungus had been growing, was shown to reproduce the silvering in young, but not mature, apple leaves (Miyairi et al. 1977, 1985; Miyairi 1987). The action of endopolygalacturonase on young leaves is consistent with the low methoxy level of young leaves (Miyairi et al. 1997b), i.e. *in vivo* silvering in mature leaves could be due to the presence of fungal pectinases able to degrade methoxylated pectins which are found in higher levels in mature leaves. These activities have not been described for *C. purpureum*, although their presence in related fungi are well-documented (Hasui et al. 1976; Nozaki et al. 1997).

To understand the role of extracellular enzyme activity in pathogenesis and system disease symptoms of silverleaf infections, the fungus was grown on media supplemented with different wood constituents, each potential substrates for the fungus *in vivo*. Extracellular proteins were then extracted from the media and a range of enzyme activities were measured.

New Zealand Plant Protection 54:202-208 (2001)

MATERIALS AND METHODS

Cultures and extract preparation

The strain of *C. purpureum* SL32 "Maxi", locally isolated from *Populus maximowiczii*, was used in all experiments. The experiments used the medium of Beever (1969), except that the glucose was omitted and an alternative carbon source supplied. The carbon sources used were glucose (BDH), starch (BDH, from potato), cellulose (Whatman CC41), carboxymethylcellulose (cmc) (Sigma), citrus pectin (Sigma), sodium polygalacturonate (Sigma), Link pectin and no carbon source. Link pectin was prepared as described by Tsuyumu et al. (1985). All carbon sources were used at 1.5% concentration except glucose, which was used at 1.4%. Blocks of fungus (5 mm x 5 mm) were cut from the actively growing front of a 7 day old culture grown on the experimental agar with glucose. These blocks were grown in Petri dishes on approximately 25 ml of media set with 1.5% agar (Germantown Co., Manakau, New Zealand). A disc of PVDF, which was the diameter of the Petri dish, was placed on the surface of the agar to prevent the mycelium from entering the medium. The cultures were grown in darkness at 22°C.

Extracts were made from cultures of the specified ages (7 to 28 days); two experimental time courses were carried out, with duplicate plates for each carbon source in each time course. One Petri dish was used for each extraction; the fungus growing on top of the PVDF was scraped off using a scalpel and dried at 37°C for two days to determine the biomass dry weight. The PVDF was removed and discarded. Extracellular enzymes were extracted by thoroughly homogenising all the media in 40 ml of 20 mM sodium acetate buffer, pH 5.0, concentrating the extract to 1 ml using an Amicon ultrafiltration cell with a 3000 Da molecular weight cut off, followed by dialysis against three changes of 20 mM sodium acetate buffer, pH 5.0 and then diluted with the same buffer to give a final volume of 3.6 ml. Extracts were stored at 4°C, and were stable for several months under these conditions.

Protein and enzyme assays

For all the enzyme assays described below, activities were determined three times for each extract. The twelve activities for each carbon source were averaged to give the final result, with the standard deviation of the values given as the standard error. Protein concentration of the extracts was measured by the bicinchoninic acid method (Smith et al. 1985) using bovine serum albumin (Boehringer Mannheim) as the protein standard. Linear regression and statistical analysis of the growth data was carried out using Microcal 'Origin' Version 5.0.

Pectin degradation was assessed using four activities. Endopolygalacturonase (ePG) activity was measured by the release of reducing groups from 0.1% w/v polygalacturonic acid; reducing groups were measured by the cyanoacetamide method (Gross 1982). Pectin methylsterase (PME) was determined by measuring the decrease in absorbance at 600 nm due to acid production during de-esterification of 4% Link pectin (Zamski & Peretz 1996). The two β -trans-eliminases, pectin lyase (PnL) and pectate lyase (PaL), were measured directly by measuring product formation at 235 nm after incubation for 20 h at 37°C with 0.3% Link pectin and 0.3% sodium polygalacturonate respectively (Hasui et al. 1976).

The activities of three enzymes involved in cellulose degradation, cellulase (Cel), cellobiohydrazase (Cbh) and endo-1,4- β -glucanase (ebG), were measured by incubating the extracts at 37°C for 20 h with each of 1% cellulose powder (Whatman CC41), 1% avicel (PH101, BDH) and 0.5% carboxymethylcellulose (Sigma). The extent of hydrolysis was determined by measuring glucose release using the cyanoacetamide method.

Lignin degradation was estimated by measuring laccase, manganese peroxidase (MnPx) and lignin peroxidase (LiPx) activities. Laccase and MnPx activities were determined by measuring over 2 h at 40°C the appearance of dimethoxyphenol breakdown products at 450 nm in the presence of saturating oxygen and 2 mM hydrogen peroxide respectively (Paszczyński et al. 1988). LiPx activity was monitored at 25°C by the formation of veratrylaldehyde at 310 nm from 2 mM veratryl alcohol (Tien & Kirk 1988). The ability to measure MnPx and LiPx activity was checked by adding partially purified enzyme to the extracts.

Other enzyme activities measured were proteinase, α -amylase and xylanase. Proteinase

was measured using 5 mg/ml bipodylcasin (Molecular Probes), a fluorimetric substrate. The activity of α -amylase was measured as p-nitrophenol production in an α -glucosidase linked assay in kit form (Ceralpha, Megazyme). Xylanase activity was measured using solubilisation of the dye, 0.6% w/v 4-O-methyl-D-glucourono-D-xylan Remazol Brilliant Blue R (Sigma) (Biely et al. 1985).

RESULTS AND DISCUSSION

Growth and extracellular protein

Dry weight growth curves are given in Figure 1. Growth on the different carbon sources roughly fall into three groups: cellulose, cmc and no carbon source all had little or no growth; starch and polygalacturonate gave intermediate growth and the fungi on the remaining carbon sources grew strongly to a maximum at about 21 days. At this point the fungus completely filled the Petri dish. The maximum growth rates seen with glucose show that it was an excellent nutrient source for the fungus. The similarly high level of growth on Link pectin and pectin as compared to polygalacturonate suggests that methylation of polygalacturonate is a potent stimulator of growth, probably by induction of catabolic enzymes.

FIGURE 1: Change in dry weight of fungal biomass over time. The extracts were from silverleaf fungus grown on cellulose, cmc, no carbon source, glucose, Link pectin, pectin, polygalacturonate or starch. Data represent the average of two duplicate plates repeated over two time courses.

In addition, the amount of extracellular protein in the extracts was determined (data not shown), and compared with the dry weight of the fungus (given in Table 1). For pectin, Link pectin, cellulose and cmc there was a linear relationship between protein in the extract and fungal biomass ($P < 0.05$). For the two pectin sources this result was not unexpected, given the high growth seen for these two substrates. However, the result for the two cellulose sources, which had the lowest growth rates, was surprising. This suggests that cellulose induces the release of protein but does not induce growth. This may indicate that *in vivo* cellulose induces enzymes able to degrade other components of the cell wall as a source of energy and is not itself a source of nutrition.

TABLE 1: Linear regression of the relationship between extracellular protein and dry weight.

Substrate	Intercept	Gradient	Correlation Coefficient	Probability value
Pectin	0.1731	0.0033	0.963	0.0001
Cellulose	0.0899	0.0815	0.826	0.012
cmc	0.0521	0.02675	0.848	0.029
Link Pectin	0.1507	0.0026	0.749	0.032
PGA	0.1115	0.0036	0.410	0.316
Glucose	0.0747	0.0003	0.194	0.646
Starch	0.1122	0.0004	0.068	0.873
Control	0.0113	-0.0004	-0.010	0.982

Enzyme activities

The specific activities of most of the enzymes tested are given in Tables 2 & 3. Enzyme activities not included were laccase, which was at a background level in all extracts and proteinase and α -amylase. For the latter two enzymes no activity was seen until day 14, and after that time all extracts had similar specific activities.

Four pectin degrading enzymes were analysed. All four activities were induced by pectin, Link pectin and to a lesser extent by polygalacturonate. They were also induced by the celluloses and starch. This is the first report of pectin lyases (β -trans eliminases) being produced by *C. purpureum* and establishes the presence of significant pectinase activity at neutral pH values. The induction of all the enzymes by Link pectin is understandable, as is the induction by polygalacturonate, especially the lesser induction of PME and pectate lyase which act on methylated pectin. Induction of pectinases during growth on pectin has been reported for the fungus *Alternaria mali* (Hasui et al. 1976), but the phenomenon has not been previously reported for *C. purpureum*. Miyairi et al. (1997a) have demonstrated the production of PME by *C. purpureum* growing on 2% pectin, and the ePG gene has recently been cloned (Miyairi et al. 1997c) as an initial step to understanding its induction.

The induction of pectinases by cellulose and cmc has not been previously reported. This induction probably partially accounts for the strong relationship between extracellular protein release and growth. The induction may be linked to the strong growth seen with pectin and Link pectin: cellulose and pectin polysaccharides are usually found intimately associated in plant cell walls, with cellulose being more abundant. Thus if silverleaf fungus was able to utilise pectin as a carbon source, but release of the pectin degrading enzymes was triggered by cellulose as well as by pectin, then it would increase the chance of successful infection.

Xylanase activity was also induced by cellulose and cmc. The combination of xylanase and ebG induced together hints that the xyloglucan network, a major cell wall fraction of dicotyledons (McCann et al. 1992), is attacked. This suggests that in the presence of cellulose a range of enzymes able to degrade the major sugar polymers in cell walls, celluloses, pectins and xyloglucans, are induced to facilitate growth. However, unlike the pectin degrading enzymes, xylanase was not induced by polygalacturonate, Link pectin or pectin.

Very little lignolytic activity was detected. Laccase activity was detected with little consistency except on the polygalacturonate substrate. MnPx was only seen in few samples that had high laccase activity, and LiPx was not detected. Proteinase and α -amylase activities were not observed until day 14, but after that time the specific activity for each enzyme was similar for all carbon sources.

The results are consistent with the pectinase and cellulase enzyme complexes playing an important role in mycelial movement through and between plant cells. This is

TABLE 2: Specific activities of endopolygalacturonase (ePG), pectin methylesterase (PME), pectate lyase (PaL), pectin lyase (PnL) and xylanase (Xyl). All activities are in mg product produced/min/mg fungus biomass. Values are the average of three estimations for each duplicate plate of two time courses, with the standard error shown in brackets.

Substrate	Time (days)	ePG	PME	PaL	PnL	Xylanase
Control	7	0.6 (0.1)	0.7 (0.4)	1.3 (0.1)	1.9 (0.5)	27 (2.0)
	14	0.8 (0.1)	1.8 (1.8)	0.1 (0.1)	9.7 (6.8)	82 (14)
	21	0.2 (0.1)	0.9 (0.9)	0.0 (0.1)	8.0 (0.8)	35 (32)
	28	0.6 (0.4)	5.1 (2.3)	0.3 (0.3)	3.0 (1.7)	68 (33)
Cellulose	7	1.7 (0.5)	2.3 (1.9)	4.8 (2.3)	5.1 (1.9)	208 (10)
	14	4.5 (2.3)	28.6 (7.2)	2.7 (0.9)	22 (13)	430 (240)
	21	0.4 (0.2)	1.8 (0.7)	0.3 (0.1)	1.7 (0.6)	27 (12)
	28	0.7 (0.1)	2.0 (0.2)	0.7 (0.3)	2.8 (1.1)	21 (3.3)
Carboxy-methyl-cellulose	7	4.4 (2.6)	1.8 (1.8)	2.7 (1.5)	3.6 (1.6)	50 (110)
	14	3.9 (0.5)	0.0 (0.1)	0.4 (0.1)	22 (8.5)	470 (33)
	21	1.5 (0.7)	0.0 (0.2)	0.0 (0.1)	11.7 (5.0)	200 (90)
	28	3.8 (0.7)	22.2 (12)	0.3 (0.3)	29 (1.1)	700 (80)
Glucose	7	0.1 (0.1)	0.4 (0.3)	1.1 (0.8)	0.3 (0.2)	2.0 (0.1)
	14	0.1 (0.2)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	2.1 (0.1)
	21	0.0 (0.2)	0.0 (0.3)	0.0 (0.2)	0.0 (0.1)	0.5 (0.1)
	28	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.3 (0.1)
Link Pectin	7	0.3 (0.1)	1.6 (0.4)	8.2 (6.5)	4.7 (1.7)	2.7 (0.2)
	14	0.0 (0.1)	0.2 (0.1)	0.3 (0.1)	0.6 (0.1)	0.0 (0.1)
	21	0.0 (0.1)	0.1 (0.1)	0.2 (0.3)	0.4 (0.1)	0.3 (0.1)
	28	0.0 (0.1)	0.1 (0.1)	0.4 (0.1)	0.5 (0.1)	0.2 (0.1)
Pectin	7	1.6 (1.0)	4.8 (3.0)	29 (16)	23 (15)	16 (10)
	14	0.0 (0.1)	0.1 (0.1)	0.3 (0.1)	0.6 (0.1)	0.3 (0.1)
	21	0.0 (0.1)	0.0 (0.2)	0.2 (0.1)	0.4 (0.1)	0.3 (0.1)
	28	0.0 (0.1)	0.0 (0.3)	0.4 (0.1)	0.4 (0.1)	0.5 (0.1)
Poly-galacturonic Acid	7	2.2 (1.4)	2.6 (0.6)	13 (5.0)	9.0 (4.1)	15 (11)
	14	0.5 (0.4)	0.1 (0.1)	12 (9.1)	3.9 (3.0)	1.6 (0.1)
	21	0.1 (0.1)	0.4 (0.1)	2.1 (0.1)	1.1 (0.2)	1.0 (0.1)
	28	0.2 (0.1)	0.4 (0.2)	3.3 (0.2)	2.5 (2.0)	2.6 (0.2)
Starch	7	3.9 (2.3)	0.5 (0.5)	0.8 (0.1)	0.2 (0.1)	7.5 (5.5)
	14	0.1 (0.1)	0.0 (0.1)	1.0 (0.1)	0.2 (0.1)	1.1 (0.1)
	21	0.1 (0.1)	0.4 (0.2)	0.7 (0.2)	0.7 (0.4)	0.0 (0.1)
	28	0.1 (0.1)	0.3 (0.1)	2.0 (1.0)	0.9 (0.6)	1.2 (0.1)

particularly true of the cellulases. The fungus grows very poorly on cellulose and carboxymethyl cellulose, and a role in intercellular penetration rather than nutrition, is consistent systemic disease symptoms. These symptoms include the silvering that ePG has been shown to cause only in young leaves (Miyairi 1987). The results in this paper suggest that cellulases may also be involved in the formation of silvering, acting in concert with ePG to affect mature tissue.

The absence of a broad spectrum of constitutently-expressed wood degrading activity correlates with observations that the silverleaf fungus tends to penetrate xylem tissues without extensive maceration of cells, and without rapid deterioration of the wood structure. This is clearly a requirement for systematic transport of phyllotoxin substance from the extracellular secretion of the fungus. The suggestion that the fungus might achieve this by pectin degradation, rather than cellulose degradation, is consistent with

TABLE 3: Specific activities of cellulase (Cel), cellobiohydraz (Cbh), endo- β -galacturonase (ebG), laccase and manganese peroxidase (MnPx). All activities are mg product produced/min/mg fungus biomass, except laccase and MnPx which are ng product produced/min/mg fungus biomass. Values are the average of three estimations for each duplicate plate of two time courses, with the standard error shown in brackets.

Substrate	Time (days)	Cel	Cbh	ebG	Laccase	MnPx
Control	7	1.1 (0.7)	0.1 (0.9)	2.5 (1.0)	2.7 (0.2)	0.0 (0.1)
	14	1.2 (0.7)	0.0 (0.1)	15.2 (6.9)	50 (48)	2.2 (1.9)
	21	0.0 (0.2)	0.4 (0.2)	9.9 (7.5)	1.0 (0.1)	0.0 (0.1)
	28	0.1 (0.1)	0.1 (0.1)	6.7 (5.6)	1.0 (0.1)	0.0 (0.1)
Cellulose	7	1.4 (1.3)	0.6 (0.1)	26.6 (1.0)	1.4 (1.2)	0.2 (0.1)
	14	1.3 (1.0)	1.6 (1.3)	29.3 (2.2)	43 (48.0)	1.9 (1.7)
	21	0.2 (0.1)	0.2 (0.2)	5.0 (2.2)	3.0 (0.1)	0.1 (0.1)
	28	0.5 (0.1)	0.4 (0.1)	3.8 (0.6)	1.0 (0.1)	0.2 (0.1)
Carboxy-methyl-cellulose	7	8.2 (4.7)	2.8 (1.7)	52.5 (31)	0.0 (0.1)	0.1 (0.1)
	14	1.3 (0.5)	1.2 (0.1)	38.1 (7.3)	0.0 (0.1)	4.7 (1.2)
	21	0.5 (0.6)	1.5 (0.7)	27.2 (10)	0.1 (0.0)	0.4 (0.2)
	28	1.3 (0.8)	2.0 (0.2)	88.6 (2.7)	0.0 (0.1)	0.2 (0.3)
Glucose	7	0.3 (0.1)	0.0 (0.1)	0.6 (0.2)	1.3 (0.2)	0.2 (0.1)
	14	0.0 (0.1)	0.0 (0.1)	0.2 (0.1)	0.8 (0.6)	0.1 (0.1)
	21	0.0 (0.1)	0.0 (0.1)	0.1 (0.1)	0.0 (0.1)	0.0 (0.1)
	28	0.0 (0.1)	0.0 (0.1)	0.1 (0.1)	0.0 (0.1)	0.0 (0.1)
Link Pectin	7	0.6 (0.1)	0.2 (0.1)	0.2 (0.1)	0.0 (0.1)	0.1 (0.2)
	14	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)
	21	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.3)
	28	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.2 (0.1)
Pectin	7	4.0 (3.4)	3.2 (3.1)	3.1 (1.7)	0.4 (0.1)	0.1 (0.1)
	14	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.1 (0.2)
	21	0.0 (0.1)	0.0 (0.1)	0.1 (0.0)	0.0 (0.1)	0.0 (0.2)
	28	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)
Poly-galcturonic Acid	7	5.3 (3.2)	1.8 (1.3)	3.6 (2.4)	0.1 (0.1)	0.1 (0.1)
	14	0.6 (0.5)	0.0 (0.6)	0.8 (0.4)	1.4 (1.1)	0.0 (0.1)
	21	0.1 (0.1)	0.0 (0.1)	0.1 (0.0)	1.3 (0.8)	0.0 (0.1)
	28	0.2 (0.1)	0.0 (0.2)	0.1 (0.1)	4.5 (2.4)	0.0 (0.1)
Starch	7	8.8 (5.0)	4.7 (5.3)	8.0 (0.8)	5.6 (4.9)	0.1 (0.1)
	14	0.0 (0.1)	0.0 (0.1)	0.1 (0.1)	3.0 (2.9)	0.0 (0.1)
	21	0.0 (0.1)	0.0 (0.1)	0.1 (0.1)	0.9 (0.6)	0.0 (0.1)
	28	0.0 (0.1)	0.0 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)

the postulated roles for the two polysaccharide networks: compression-resistance and stretch-resistance respectively (McCann & Roberts 1994). Whether the induced systemic disease is serendipitous or gives an advantage to the fungus in its growth and completion of its life cycle is not known.

ACKNOWLEDGEMENTS

We wish to thank Dr R Farrell (Waikato University) for supplying the samples of partially purified *Phanerochaete chrysosporium* LiPx and MnPx. Funding was provided by the New Zealand Foundation for Research, Science and Technology (Contract CO6516).

REFERENCES

- Beever, D.J. 1969: Nutrition of the silverleaf pathogen *Stereum purpureum* (Pers.) Fr. *N.Z. J. Sci.* 12: 268-275.
- Biely, P.; Mislovicová, D.; Toman, R. 1985: Soluble chromogenic substrates for the assay of endo-1,4- β -xylanases and endo-1,4- β -glucanases. *Anal. Biochem.* 144: 142-146.
- Gross, K.C. 1982: A rapid and sensitive method for assaying polygalacturonase using 2-cyanoacetamide. *HortScience.* 17: 933-934.
- Hasui, Y.; Miyairi, K.; Okuno, T.; Sawai, K.; Sawamura, K. 1976: Purification and properties of pectin *trans*-eliminase from *Alternaria mali*. *Annals Phytopath. Soc. Japan* 42: 228-231.
- McCann, M.C.; Roberts, K. 1994: Changes in cell wall architecture during cell elongation. *J. Exp. Bot.* 45: 1683-1691.
- McCann, M.C.; Wells, B.; Roberts, K. 1992: Complexity in the spatial localization and length distribution of plant cell-wall matrix polysaccharides. *J. Microscopy* 166: 123-136.
- Miyairi, K.; Fujita, K.; Okuno, T.; Sawai, K. 1977: A toxic protein causative of silverleaf disease symptoms on apple trees. *Agric. Biol. Chem.* 41: 1897-1902.
- Miyairi, K.; Hamada, S.; Okuno, T. 1997a: Purification and structural characterization of PE I and PE II produced by *Stereum purpureum*. *Bulletin of the Faculty of Agriculture, Hirosaki University* (In Japanese) 61: 15-24.
- Miyairi, K.; Ito, K.; Okuno, T. 1997b: Preparation and Characterization of pectic polysaccharides from silvered leaves of apple trees infected with *Stereum purpureum*. *Bulletin of the Faculty of Agriculture, Hirosaki University* 61: 1-14.
- Miyairi, K.; Okuno, T.; Sawai, K. 1985: Purification and properties of endopolygalacturonase I from *Stereum purpureum*, a factor inducing silver-leaf symptoms in apple trees. *Agric. Biol. Chem.* 49: 1111-1118.
- Miyairi, K.; Senda, M.; Watanabe, M.; Hasui, Y.; Okuno, T. 1997c: Cloning and sequence analysis of cDNA encoding endopolygalacturonase I from *Stereum purpureum*. *Biosci. Biotech. Biochem.* 61: 655-659.
- Miyairi, K. 1987: Silver-leaf inducing substance of apple silver-leaf disease. *Kagaku To Seibutu* (In Japanese) 25: 288-290.
- Nozaki, K.; Miyairi, K.; Hozumi, S.; Fukui, Y.; Okuno, T. 1997: Novel exopolygalacturonases produced by *Alternaria mali*. *Biosci. Biotech. Biochem.* 61: 75-80.
- Paszczynski, A.; Crawford, R.L.; Huynh, V-B. 1988: Manganese peroxidase of *Phanerochaete chrysosporium*. *Methods in Enzymology* 161B: 264-270.
- Pearce, R.B.; Sumer, S.; Doran, S.J.; Carpenter, T.A.; Hall, L.D. 1994: Non-invasive imaging of fungal colonization and host response in the living sapwood of sycamore (*Acer pseudoplatanus* L.) using nuclear magnetic resonance. *Physiol. Plant Path.* 45: 359-384.
- Smith, P.K.; Krohn, R.I.; Hermanson, G.E.; Mallia, A.K.; Gartner, F.H.; Provenzano, M.D.; Fujimoto, E.K.; Goeke, N.M.; Olson, B.J.; Klenk, D.C. 1985: Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76-85.
- Spiers, A.G.; Edwards, W.R.N.; Hopcroft, D.H. 1987: Effects of silverleaf infection on the ultrastructure of foliage of *Prunus*, *Rosa* and *Populus*. *N.Z. J. Bot.* 25: 411-422.
- Spiers, A.G.; Hopcroft, D.H. 1988: Factors affecting *Chondrostereum purpureum* infection of *Salix*. *European J. Forest Path.* 18: 257-278.
- Tien, M.; Kirk, K. 1988: Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods in Enzymology* 161B: 238-248.
- Tsuyumu, S.; Funakubo, T.; Chatterjee, A.K. 1985: Purification and partial characterisation of a mitomycin C-induced pectin lyase from *Erwinia chrysanthemi* strain EC 183. *Physiol. Plant Path.* 27: 119-130.
- Zamski, E.; Peretz, I. 1996: Cavity spot of carrots: II. Cell-wall-degrading enzymes secreted by *Pythium* and pathogen-related proteins produced by the root cells. *Ann. Appl. Biol.* 128: 195-207.