NISEB JOURNAL Vol. 11, No. 1 , January 31, 2011
Printed in Nigeria

1595-6938/2011 $12.00 + 0.00

NISEB 2010056/11101

Cell Wall degrading enzyme activities in plant-bacteria Interaction involving Dasheen (*Colocasia esculenta* (L.) Schott) *in vitro*

Odette S. Knight [a], Helen N. Asemota[a] and Antony U. Osagie*[b]

[a]Department of Basic Medical Sciences, University of the West Indies, Mona Kingston 7, Jamaica, W.I.
[b]Biochemistry Unit, Faculty of Basic and Applied Sciences, Benson Idahosa University, P.M.B. 1100, Ugbor Road, GRA, Benin City, Edo State, Nigeria

(Received August 28, 2010; Accepted December 11, 2010)

ABSTRACT: The cell wall degrading enzymes pectin methylesterase, polygalacturonase, cellulase and amylase were monitored during the pathogenicity study of Cocoyam or dasheen (*Colocasia esculenta* (L.) Schott var *esculenta*) with *Pseudomonas sp*. All the enzymes were detected in the shoots and roots of the infected and the uninfected *in vitro* derived plantlets at the start. There was a significant increase ($P < 0.01$) in the activities of two (2) enzymes in the shoots of the infected plantlets as the infection progressed towards eighteen days when assessed by the One Way Analysis of Variance. The activities of polygalacturonase, cellulase and amylase were found to be significant ($P < 0.01$) in the roots of the infected plantlets relative to the control (uninfected plantlets). These results suggest that the synergistic interactions of these cell wall degrading enzymes facilitated the development of disease symptoms in the *in vitro* dasheen plantlets and the furtherance of the infection by the *Pseudomonas sp* in the inoculated plantlets. The infection of dasheen caused by *Pseudomonas* sp could possibly be another disease limiting the increased production of this tuber crop.

Key words: *Colocasia esculenta* (L.) Schott, pathogenicity, in vitro, cell wall degrading enzymes, *Pseudomonas sp*

Introduction

Colocasia esculenta (L.) Schott dasheen or (cocoyam) is largely cultivated throughout the humid tropics and sub-tropics for its edible corms and leaves (Lee, 1999). Dasheen tubers are consumed boiled, baked, roasted, or fried, and the young leaves and petioles eaten as vegetable either blanched or in soup. It is an important source of carbohydrate and vitamins and contains low levels of proteins, fats and fibres. The carbohydrate is readily digested when used for food, making it an ideal specialty food for potentially gluten allergic infants and people with gastrointestinal disorders (Kay, 1987). The cultivation of cocoyam in many parts of the world occurs with no major disease problems. However, in other geographic locations, diseases cause significant economic losses and have occasionally resulted in the abandonment of cocoyam cultivation. Corm rots cause considerable damage but viral diseases and that of leaf blight are most devastating in some locals (Centre for Overseas Pest Research, 1982).

*To whom correspondence should be addressed.

Review of literature has indicated no reported incidences of severe pathogenic disease implicating *Pseudomonas* sp as phytopathogens of dasheen. However, incidences of bacterial disease involving *Colocasia esculenta* (L.) Schott and other members of the araceae genera have been cited. Chase *et al*, 1992, indicated that one of the most serious diseases affecting members of the Araceae family (aroids) is caused by strains of *Xanthomonas campestris*. Xanthomonas diseases of aroids are characterized by marginal chlorosis and necrosis, interveinal necrosis, and in some cases a systemic infection resulting in plant death. The successful invasion of a plant's cell wall and the degradation of its components or the digestion of the plant material to facilitate the progress of disease development are also attributed to the phytopathogen's ability to secrete cell degrading enzymes (Misaghi, 1982).

The occurrence of cell wall degrading enzymes in many plant-pathogen interactions prompted many investigators to study the function of these extracellular enzymes in infection processes (reviewed in Walton, 1994; Hugouvieux-Cott-Pattat *et al*, 1996; Annis and Goodwin, 1997; Py *et al*, 1998). Daniels *et al*, 1988 indicated that phytopathogens produce a range of these enzymes, including but not limited to, pectinases, cellulases, proteases, amylases, and phospholipases which may have a considerable part to play in the development of symptoms and pathogenesis. Penetration of pathogens into parenchymatous tissues is brought about by the breakdown of the cell walls, which consist of cellulose, pectins, hemicelluloses, structural proteins, and of the middle lamellae, which consists primarily of pectins. The degradation of each of these substances is brought about by the action of one or more sets of enzymes secreted by the pathogen. This paper reports the detection and variation in activities of pectin methylesterase, polygalacturonase, cellulase and amylase during the controlled *in vitro* pathogenicity study, as indicators of the establishment of a plant-bacteria interaction between dasheen and *Pseudomonas* sp.

Materials and Methods

In vitro derived Dasheen plantlets

In vitro derived dasheen plantlets, cultured from the apical meristem of young dasheen shoots, were initiated and multiplied on Murashige and Skoog basal salt mixture, MS (Murashige Skoog, 1962), pH 5.8 containing 30g/L sucrose, 0.5 mg/L (for initiation) and 0.1 mg/L (for multiplication) benzene amino purine (BAP). At both the initiation and multiplication stages in vitro dasheen cultures were transferred to the tissue culture growth room of the Biotechnology centre, University of the West Indies, which has a 16 hour photoperiod at 24-27^{0}C (Mitchell *et al*, 1994). Mature plantlets, approximately six (6) months old were used in the pathogenicity study.

Isolation and Identification of Pseudomonas sp

The pathogenic bacteria were isolated from samples of infected dasheen leaves collected from a dasheen cultivation located in Sligoville, St. Catherine, Jamaica, W.I. These bacteria were cultured microbiologically on Nutrient Yeast Glycerol Agar [NYGA], 5 g of Bacto Peptone (Difco Laboratories, Detroit, Michigan) per litre, 3 g of yeast extract per litre, 20 g of glycerol per litre and 18 g of Bacto Agar per litre] plates as indicated by Bradbury (1970). Typical yellow-mucoid, pure colonies, which were motile, Gram-negative rods were identified as Pseudomonas sp through 16SrRNA Gene Sequence Similarity.

Infection of Dasheen with Pseudomonas sp

Overnight broth cultures of the bacteria isolates were used to inoculate the leaves of these tissue culture plantlet. For the inoculation, a sterile needle was used to create wounds at least 1 cm apart on the leaf's top surface at the same point where sterile cotton previously soaked in broth culture was placed on the underside. Samples were collected at day 6, 12 and 18 and assayed for the different enzyme activities. For the enzymes assays the shoots and roots of both the control and the infected plantlets were weighed, homogenized and extracted at 4^{0}C in the appropriate quantity of buffer. The homogenate was centrifuged for 20 minutes at 8,000 rpm and the resulting suspension served as the source of enzymes for the different assays.

Extraction of three (3) cell wall degrading enzymes

Pectin methylesterase, polygalacturonase and cellulose were extracted from weighed samples of infected and uninfected (control) cocoyam/ dasheen plantlets. The samples were homogenized using 5 mls of 0.2 M sodium

phosphate buffer, pH 6.8 per gram of sample. The resulting suspension was centrifuged for 20 minutes at 8,000 rpm and 4^0C, and the supernatant or appropriate dilution, as required was used in the assay of the enzymes.

Assay of Polygalacturonase

Polygalacturonase activity was determined by the method of Bateman *et al* (1969). A 0.5 ml volume of the enzyme extract and 0.5 ml of 1.0 g litre^{-1} sodium polypectate in 0.2 M sodium phosphate buffer, pH 6.8 were incubated in a test tube (triplicate) at 30^0C. After 30 min the tubes were transferred to a boiling water bath for 5 min to stop the reaction. The method of Nelson (1944) as modified by Somogyi (1952) was used to assay reducing sugars. The equivalent galacturonate released was read off a calibration curve and polygalacturonase activity was expressed as milligram galacturonate produced per minute per milligram protein.

Assay of Cellulase

The assay of cellulase was also executed using the method of Bateman *et al* (1969). A 0.5 ml portion of the enzyme extract and 0.5 ml of 1.0 g litre $-^1$ carboxymethylcellulose (CMC) in 0.2 M sodium phosphate buffer, pH 6.8 were incubated in a test tube (triplicate) at 30^0C. After 30 min, 1.0 ml 2 M sodium hydroxide was used to terminate the reaction. The method of Nelson (1944) as modified by Somogyi (1952) was used to assay reducing sugars. Glucose released was read off a calibration curve and cellulose activity was expressed as milligram glucose produced per minute per milligram protein.

Assay of pectin methylesterase

The assay of pectin methylesterase was elucidated by the procedure of Rouse and Atkins (1955) with slight modifications by Okolie and Ugochukwu (1988). An enzyme extract of 0.5 ml was transferred to triplicate boiling tubes containing 1.25 ml pectin (1 g litre $^{-1}$ in 0.1 M sodium chloride). The reaction mixture was then incubated at 30^oC for 30 min. After incubation, the tubes were placed in a boiling water bath for 5min to terminate the reaction. The tubes were cooled to room temperature (25^oC) and 3 drops of 1.0 g litre $^{-1}$ phenolphthalein in 590 ml litre $^{-1}$ ethanol were added to the mixture; it was then titrated against 0.02 M sodium hydroxide to a faint pink colour. Pectin methylesterase activity was then calculated and expressed as the ester equivalent hydrolyzed per minute per gram sample. One equivalent of ester was defined as that whose hydrolysis requires 0.01 ml of 0.02 M sodium hydroxide for neutralization of the free – COOH groups produced under the assay conditions.

Extraction and assay of amylase

Amylase was extracted and assayed by a slightly modified procedure of Davies and Ross (1987). Control and infected samples of shoots and roots of C. *esculenta* were weighed and homogenized with 1 g 5 millilitre $^{-1}$ of 0.1 M citrate-phosphate buffer, pH 6.0 containing 20 mM calcium chloride. The homogenate was centrifuged at 4^0C for 20 minutes at 8,000 rpm. The supernatant subsequently served as a source of both α and β amylase. The assay mixture (in triplicate) contained 5 mg millilitre -^{1} of starch, 0.1 M sodium fluoride, 0.2 M sodium acetate buffer pH 4.8., and 1 ml of enzyme extract. The tubes were then incubated at room temperature (25^0C) for 15 minutes; then 2 ml of 2 M sodium hydroxide was added to terminate the reaction. The reducing sugar produced was measured by the method of Nelson-Somogyi as described by Plummer (1978).

Statistical Analysis

The significance of the enzyme activity was determined by the use of the One Way Analysis of Variance (ANOVA).

Results and Discussion

A brown colouration was detected around the wounds created on the leaves of infected dasheen plantlets 4 to 6 days following the inoculation of the leaves with *Pseudomonas sp*. These areas became rapidly necrotic and coalesced to produce larger areas of dead tissue. During days 6 to 12 the entire leaf became a soaked brown mass due to severe necrosis. In the last 6 days of the infection, that is, days 12 to 18 the severe necrosis seen in the leaf

had spread to the petioles causing loss of structural coherence and the subsequent death of this organ. It was difficult to visually detect any physiological changes in the infected roots. Pectin methylesterase (PME) activity was detected in shoots and roots of infected and uninfected dasheen plantlets. There was a significant difference ($P< 0.01$) between the activity levels of PME in the shoots of the infected versus the uninfected plantlets. However, the difference between the PME levels in the roots of infected and uninfected plantlets was not (Figure 1). Literature has indicated that phytopathogenic microorganisms seem to produce at all times small, constitutive, base-level amounts of pectolytic enzymes that, in the presence of pectin, release from it a small number of galacturonan monomers, dimers or oligomers. These molecules, when absorbed by the pathogen, serve as inducers for enhanced synthesis and release of additional pectolytic enzymes, which further increase the amount of galacturonan monomers, dimers or oligomers. These simple sugars are readily assimilated by the pathogen; however, at higher concentration they act to repress the synthesis of these same enzymes, thus reducing production of the enzymes and subsequent release of additional galacturonan monomers.

The production of pectolytic enzymes is also repressed when the pathogen is grown in the presence of glucose (Agrios 1997). It was observed that PME's activity in the shoots of the infected plantlets increased by approximately 2 ½ times its initial activity on day 6, but fell to a low of 3 ester equivalent hydrolysed min $^{-1}$ gram $^{-1}$ sample, however, again increasing on day 18 by 7 units to another high (similar to that of day 6) of about 11 ester equivalent hydrolysed min $^{-1}$ gram $^{-1}$ sample. Noticeably, an increase in the PME levels of the shoots of infested plantlets coincided with the symptoms of tissue softening, progressive spread of necrosis in the petioles and the loss of structural coherence that occurred between days 12 to 18. This increase in PME in infected tissue was expected, as Demain and Phaff, 1957 indicated that PME's though present in healthy tissues of many plant species, are found in larger amounts in diseased tissue. It can therefore be suggested that the increases in PME at days 6 and 18 were brought about by the pathogen's secretion of this enzyme (Bateman and Miller, 1966). However, the drastic reduction seen at day 12 could have been caused by catabolic repression where the increase in available substrate (galacturonan monomers, dimers or oligomers) inhibits the further production of PME. For effective degradation to occur PME has to function in conjunction with chain-splitting pectinases (such as polygalacturonase) as it can only remove small branches from the pectin chain, preparing it for further attack by these chain-splitting pectinases.

The role of PME in this plant-bacteria interaction was therefore significant to the development of the disease in the shoots of the infected plantlets. The activity of PME in the roots of infected plantlets was not considered significantly different from the activity in the uninfected plantlets. Bateman and Millar (1966) indicated that the nature of the chemical and physical associations of galacturonide and non-galacturonide constituents of the cell wall would influence the extent to which pectic substances undergo enzymatic degradation in plant tissue. It must be noted that the nature of these plant-bacteria associations may be expected to vary from plant species to species, from one plant tissue to another, and within the same tissue depending upon its nutritional status and age. Therefore there are grounds supporting the variation in the level of low PME activity seen in the roots versus the increased activity observed in the shoots.

Polygalacturonase (PG) activity was present in infected and control dasheen plantlets at the initial stage of the pathogenicity study with *Pseudomonas* sp (Figure 2). The activities of PG in both the shoots and roots were significantly different ($P < 0.01$) in the infected plantlets as against the activities in the uninfected plantlets. The pattern of PG's activity in the shoots of the infected plantlets was similar to that of PME, in that, there was an increase at day 6, followed by a significant decrease at day 12, and then an increase at day 18. For polygalacturonase to bring about any significant hydrolysis of pectin components in the cell wall, it requires the partial demethylation of pectin, which is carried out by pectin methylesterase (Jansen and Jang, 1960; McCready and McComb, 1955; Brady, 1976). Polygalacturonases are chain-splitting pectic glycosidases. The action of PG involves the addition of a molecule of water causing the breaking (hydrolyzing) of the bond between two galacturonan molecules. Considering the limited distribution of polygalacturonases in higher plants (Barash and Khazzam, 1970) it can be suggested that the polygalacturonase that played a role in the softening of the tissue and loss of coherence seen during days 12 to 18 in the infected plantlets, was produced by the Pseudomonas sp. McCready and McComb (1955) reported that polygalacturonase activity could not be detected in unripe Fuerte avocadoes, while high activity levels found in ripe fruits were directly related to the extensive decrease in the molecular size of pectin. Similarly, there was also a significant difference ($P<0.01$) in the PG activity of the infected roots, as a gradual increase in the activity in the infected plantlets versus the uninfected was observed.

Pectin, by virtue of its location mainly in the outer wall regions within the middle lamella (Mcneil *et al*, 1984) of plant cell walls, is rendered more exposed than other cell wall components, and are therefore especially susceptible to enzymatic degradation (Collmer, 1987). Therefore the actions of the pectin degrading enzymes, PME and PG have served to expose other underlying structures of the cell wall to other cell wall degrading enzymes such as cellulase and amylase (Bateman and Basham, 1976; Talmadge *et al*, 1973). Therefore PME and PG were central to

the role of penetration of the plant tissues by the Pseudomonas sp. Biochemical investigations in the fermentation-dependent softening of cassava tubers showed that cellulase, PME and PG were of key importance (Okolie and Ugochukwu, 1988). These findings are also considered consistent with the primary role of these enzymes in host/pathogen interactions, (Bateman and Millar, 1966; Byrde and Fielding, 1968) which have been shown to cause tissue maceration, electrolyte losses and cell death (Bateman and Millar, 1966; Byrde and Fielding, 1968; Goribaldi and Bateman, 1971). Studies have also shown that a positive correlation has been established between increases in polygalacturonase (Okolie and Obasi, 1992; McCready and McComb, 1955; Hultin and Levine, 1965; Raymond and Phaff, 1965; Barash and Khazzan, 1970; Zauberman and Schiffmann-Nadel, 1972; Awad and Young 1979) and cellulase (Awad 1977; Awad and Young 1979) and the softening of the avocado fruit. In the softening of many fruits PME has been shown to increase (Hobsen 1963; Hultin and Levine 1965) or decrease (Zauberman and Schiffmann-Nadel 1972; Awad and Young 1979). Additionally, Awad and Young (1972) and Zauberman and Schiffmann-Nadel (1972) have shown that an inverse relationship occurred between PG and PME activities during the ripening of avocado fruit, with softening occurring when PG was maximal and PME minimal. However, the results of this study showed that when softening of tissue occurred in dasheen plantlets, both PG and PME had increased activities in the shoots, but in the roots, while PG increased, there was little change in PME.

Both the shoots and roots of the infected and control dasheen plantlets exhibited cellulase activity at the beginning of the period of infection (Figure 3). The activity of cellulase in the roots of the infected plantlets was found to be significantly different ($P < 0.01$) from that of the uninfected plantlets. However, the difference in the activity of the shoots in both the infected and uninfected plantlets was not significant. This noticeable difference in cellulase activity between the roots and the shoots was expected as the cell walls of roots have a greater composition of cellulose than shoots considering its specialized function as a storage organ for carbohydrates. Subsequently, the cellulolytic enzymes secreted by the *Pseudomonas sp* have available glucose molecules, produced from the breakdown of cellulose, to provide nutrients to facilitate proliferation, and therefore aiding in the progression of the disease. Cellulases may further participate indirectly in disease development by releasing, from cellulose chains, soluble sugars that serve as food for the phytopathogens and, in the vascular diseases, librating into the transpiration stream large molecules from cellulose, which interfere with the normal movement of water. In general, although cellulase activity in the shoots of the infected dasheen plantlets was not much different from the uninfected plantlets, they played a role in the softening and disintegration of cell wall material of living plant tissues, facilitating the penetration and spread of the pathogen in the host and the collapse and disintegration of the cellular structure, thereby aiding the pathogen in disease production.

Amylase activity was detected in the shoots and roots of both the control and infected dasheen plantlets at the start; however the activity in the shoots was 6 times greater than that found in the roots (Figure 4). Similar to the activity of cellulase, amylase activity of the roots of infected plantlets was significantly different ($P < 0.01$) from that in the uninfected plantlets, whilst there was no significant difference in activity of both the infected and uninfected plantlets. Increases in amylase activity in roots could possibly have been due to the presence of starch, which would more or less start to develop in these 6-month old plantlets, as dasheen produces starchy underground corms. Most pathogens utilize starch and other reserve polysaccharide in their metabolic activities (Agrios, 1997). Amylase is also one of the cell wall degrading enzymes, produced by plant pathogens to facilitate the progress of disease development. Their mechanism involves the cleaving of glucosidic linkages in starch, the plant storage polysaccharide. It was shown by Okolie and Ugochukwu (1988) that amylase does not seem to play any significant part in the fermentation of cassava with *Citrobacter freundii*. In the ripening and subsequent softening of plantain *(Musa paradisiaca)*, amylase activity dramatically increased (Iyare and Ekwukoma, 1992). In light of the increase in the activity of amylase in the roots of the infected planlets, it can be deduced that the *Pseudomonas sp*. are capable of producing α- Amylases, the common form of amylase found in microorganisms as against β-Amylases (from plants) and y-Amylases, mainly associated with fungi.

The resulting disease of the plant-bacteria interaction involving dasheen was facilitated by the 4 cell wall degrading enzymes discussed above. As the infection progressed, the activity of these enzymes varied between the shoots and roots, as was expected. The shoots were more affected by activities of PME and PG, while the roots were more susceptible to attack from PG, cellulase and amylase. Therefore *Pseudomonas* sp are capable of producing cell wall degrading enzymes which facilitate the development of chlorotic and necrotic symptoms in the plant-bacteria interaction involving *in vitro* derived dasheen plantlets.

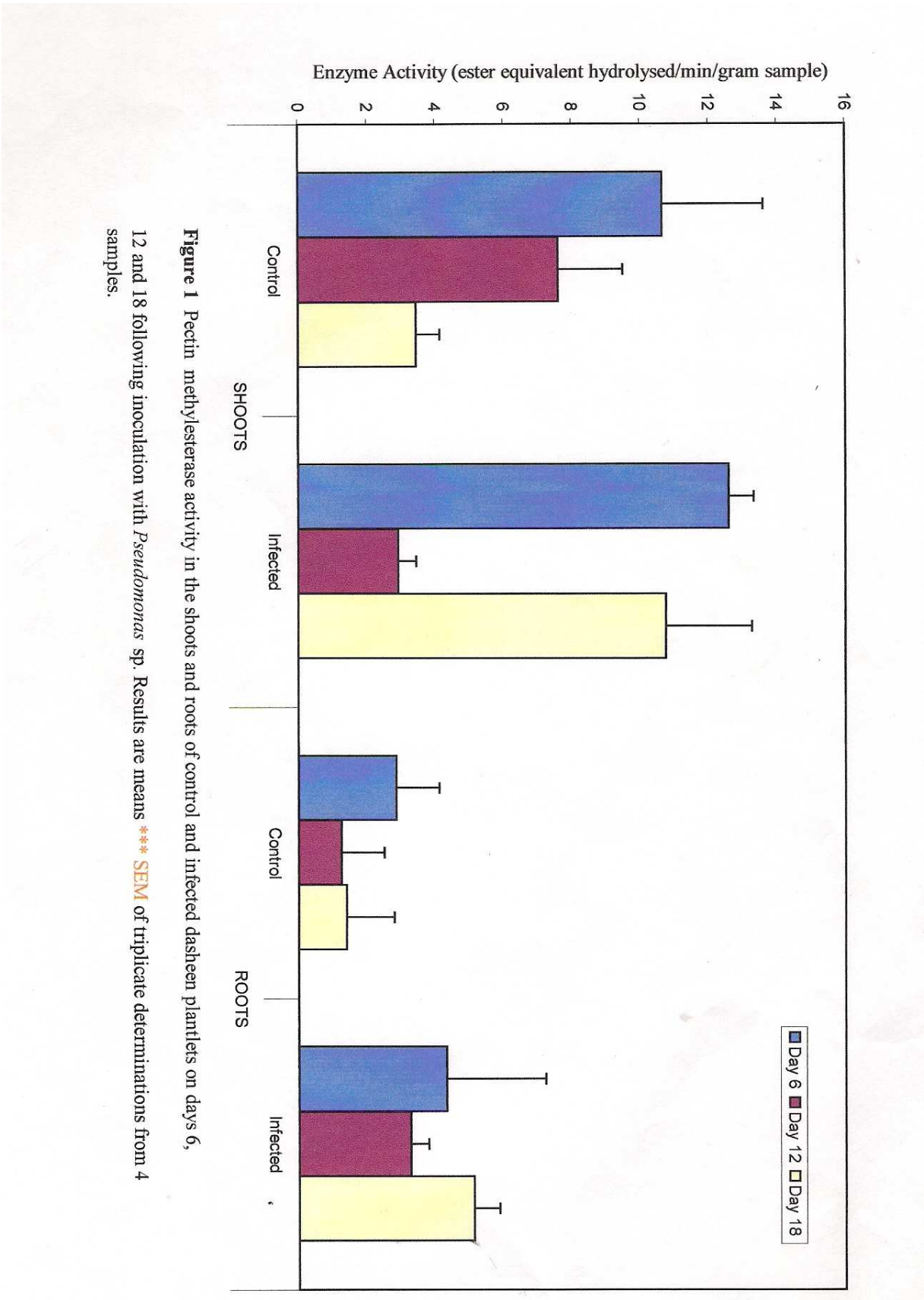

Figure 1 Pectin methylesterase activity in the shoots and roots of control and infected dasheen plantlets on days 6, 12 and 18 following inoculation with *Pseudomonas* sp. Results are means *** SEM of triplicate determinations from 4 samples.

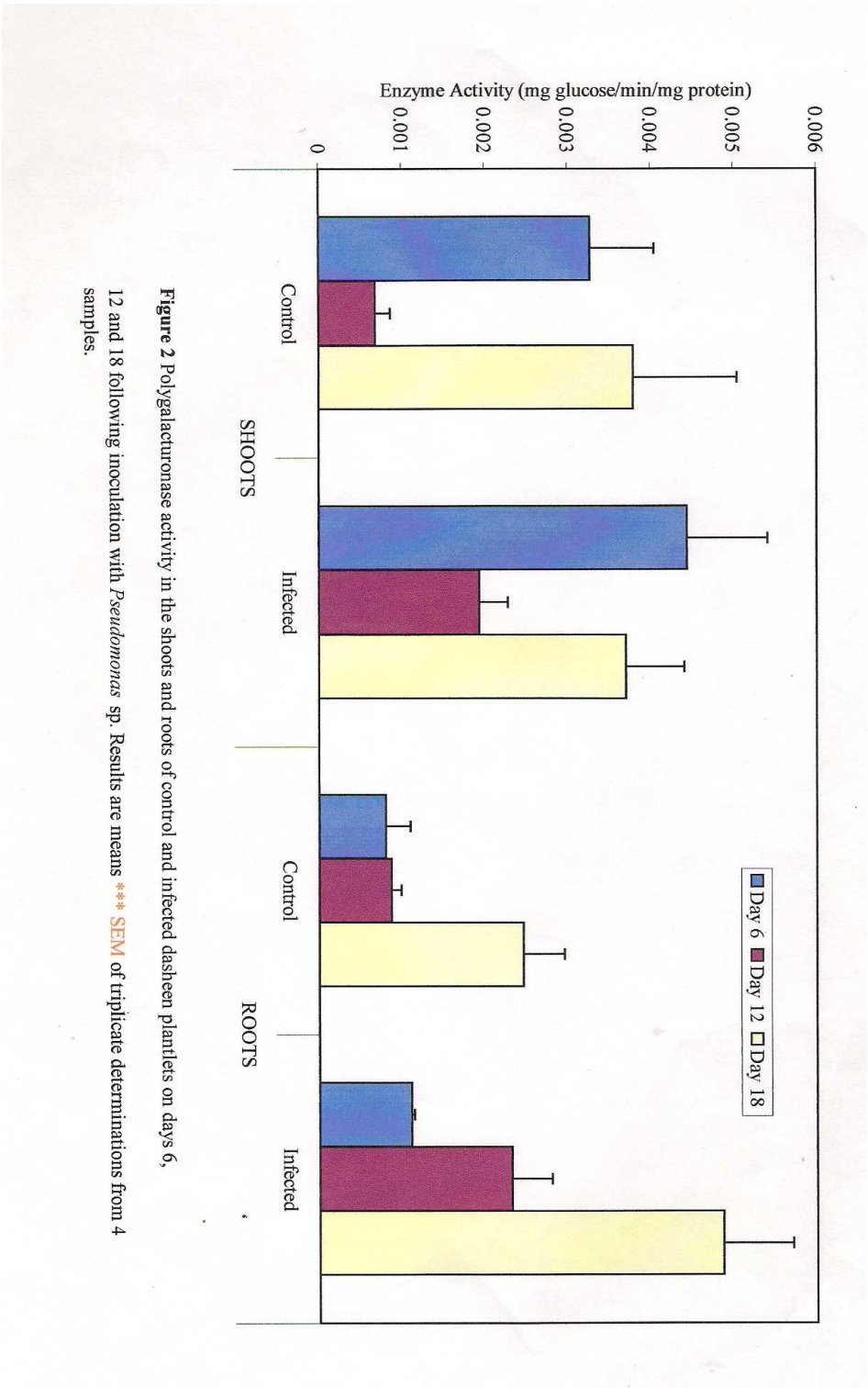

Figure 2 Polygalacturonase activity in the shoots and roots of control and infected dasheen plantlets on days 6, 12 and 18 following inoculation with *Pseudomonas* sp. Results are means *** SEM of triplicate determinations from 4 samples.

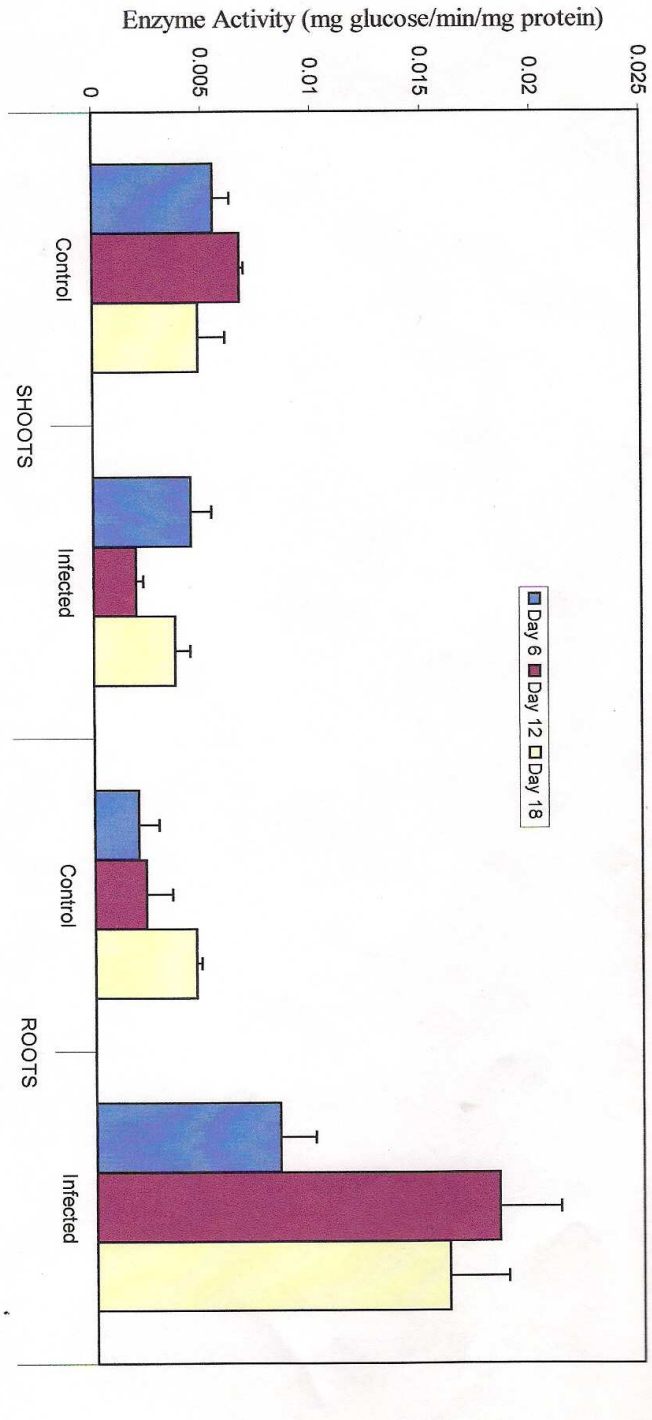

Figure 3 Cellulase activity in the shoots and roots of control and infected dasheen plantlets on days 6 , 12 and 18 following inoculation with *Pseudomonas* sp. Results are means *** SEM of triplicate determinations from 4 samples.

REr

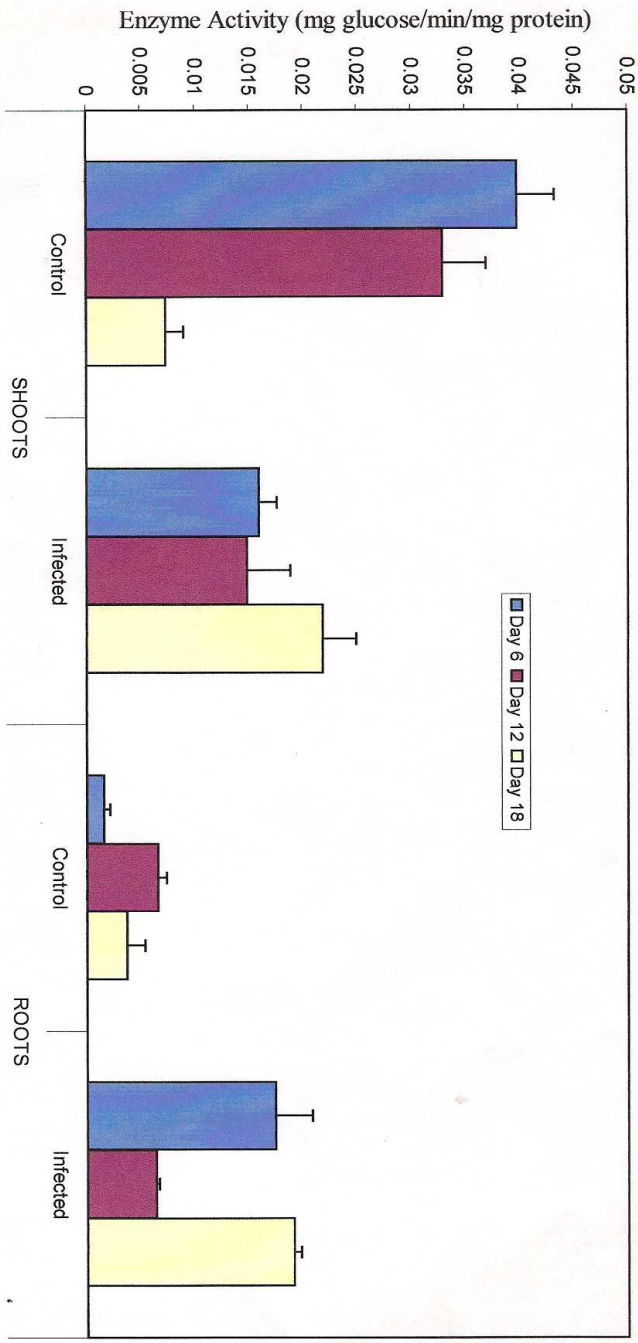

Figure 4 Amylase activity in the shoots and roots of control and infected dasheen plantlets on days 6, 12 and 18 following inoculation with *Pseudomonas* sp. Results are means *** SEM of triplicate determinations from 4 samples.

References

AGRIOS, G.N. 1997. Plant Pathology. Academic Press, San Diego, USA 609 pp

ANNIS, S.L and Goodwin, P.H. 1997. Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. Eur. J. *Plant Pathol* **103**:1-4

AWAD, M. 1977. Variation in cellulose content of Fuerte avocado fruit after harvest. *Hort Sci* **12** 406-411.

AWAD, M, and Young, R.E 1979. Post-harvest variation in cellulose, polgalacturonase and pectin methlesterase in avocado *(Persea Americana* Mill, cv. Fuerte) fruit in relation to respiration and ethylene production. *Plant physiol* **64**, 306-308

BARASH, I. and Khazzan, S. 1970. The relationship and properties of pectic glucosidases produced by host and pathogen during anthracnose disease of avocado. *Phytochemistry* **9**, 1189-1197.

BATEMAN, D.F., and Basham, H.G. 1976. Degradation of plant cell walls and membranes by microbial enzymes, p. 316-355. In R. Heitefuss and P.H. Williams (ed), Encyclopedia of plant physiology, Vol 4. Springer-Verlag, New York.

BATEMAN, D.F. and Millar, R.L. 1966. Pectic enzymes in tissue degradation. Ann Rev *Phytopathol* **4**, 119-146

BRADBURY, J.F. 1970. Isolation and preliminary study of bacteria from plants. *Rev. PI Path* **49**, 213-218.

BRADY, C.J. 1976. The pectin esterase of the pulp of the banana fruit. Aust J Plant *Physiology* **3**, 163-172.

BYRDE, R.J.W., Fielding, A.H. and Williams, A.H. 1959. The role of oxidized phenols in the varietal resistance of apples to brown rot. In Phenolics in Plants in Health and Disease, 95-99 (Pridham, J.B., Pergamon Press, New York).

Centre for Overseas Pest Research, 1982. Pest control in Tropical root crops. PANS Manual, No. 4, London.

CHASE, A.R., Stall, R.E., Hodge, N.C. and Jones, J.B. 1992. Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological, and fatty acid analyses. *Phytopathology* **82**, 754-759.

COLLMER, A. 1987. Pectic Enzymes And Bacterial Invasion Of Plants, P. 253-284. In T. Kosuge and E.W. Nester (ed), Plant-Microbe interactions: molecular and genetic perspectives, vol 11. Macmillan Publishing Co., New York.

DANIELS, M.J., Dow, M.J. and Osbourn, A.E. 1988. Molecular genetics of pathogenicity in phytopathogentic bacteria. Ann Rev Phytopathol 26: 285-312.

DAVIS D.D. and Ross 1987. Hydrolytic and phosphorolytic enzymes activity and reserve mobilization in sprouting tubers of potato *Solanum tuberosum. J Plant Physiol* **126** 387-396.

DEMAIN, A.L. and Phaff, H.F. 1957. *Walerstein Lab Commun* **20** 119.

GORIBALDI, A. and Bateman, D.F. 1971. Association of pectolytic and cellulolytic enzymes with bacteria slow wilt of carnation caused by *Erwinia Chrysanthmi. Phytopathol Med* **IX,** 136-144.

HOBSEN, G.E. 1963. Pectinesterase in normal and abnormal tomato fruit. *Biochem J* **86**, 258-365.

HUGOUVIEUX-COTTE-PATTAT, N., Condemine, G., Nasser, W. and Reverchon, S. (1996) Regulation of pectinolysis in *Erwinia chrysanthemi*. *Annu Rev Microbiol* **50**, 213-257.

HULTIN, H.O., and Levine, A.S. 1965. Pectin methylesterase in the ripening banana. *J Food Sci* 30, 917-921.

IYARE, O.A. and Ekwukoma, O. 1992. Changes in the activities of carbohydrate-degrading enzymes with ripening in *Musa paradisiaca*. J. Sci Food Agric 58, 173-176

JANSEN, E.F. and Jang, R. 1960. Orange pectinesterase binding activity. *Food Res* **25**, 64-72.

KAY, D.E. (revised by Gooding, E.G.B). 1987. Crop and Product Digest No 2- Root Crops, Second Edition. London: Tropical Development and Research Institute, XV& 380pp.

LEE, W. 1999. Ethnobotanical Leaflets: Taro (Colocasia esculenta). Southern IIinois University Carbondale. 7 Mar. 2002

URL: http://www,siu.edu/ ~ebl/leaflets/taro.htm>)

McCREADY, R.M. and McComb, E.A. 1955. Pectin constituents in ripe and unripe fruit. *Food Res* **19**, 530-535.

McNEIL, M., Darville, A.G., S.C., and Albersheim. 1984. Structure and function of primary cell walls of plants. *Annu Rev Biochem* **53**, 625-633.

MISAGHI, I.J., 1982. Physiology and Biochemistry of Plant – Pathogen Interactions. In: Chapter 3, The Role of Pathogen-Produced Cell-Wall-Degrading Enzymes in Pathogenesis. Plenum Press. New York and London, pp 17-34.

MITCHELL, S.A., Asemota, H.N. and Ahmed, M.H. 1994. Factors affecting the *in-vitro* establishment of Jamaican yam (Dioscorea spp) from nodal pieces. *J Sci Agri* **67**, 541-550.

MURASHIGE, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* **15,** 473-497.

NELSON, N.J. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol Chem* **153,** 375-380.

OKOLIE, P.N. and Obasi, B.N. 1992. Implication of cell wall degrading enzymes in heat-induced softening of the African pear (Dacryodes edulis (G Don) H J Lam). *J Sci Food Agric* **59,** 59-63.

OKOLIE, P.N. and Ugochukwu, E.N. 1988. Changes in activities of cell wall degrading enzymes during fermentation of Cassava *(Manihot esculenta Crantz)* with *Citrobacter Freundii. J Sci Food Agric* **44,** 51-61.

PLUMMER, D.T. 1978. In introduction to practical biochemistry. Second edition. McGraw Hill, Maidenhead, pp. 109-111.

PY, B., Barras, F., Harris S., Robson, N., and Salmond, G.P.C. 1998 Extracellular enzymes and their role in Erwinia virulence. In: *Methods in Microbiology*, p. 157-168. Academic Press LTD, London, UK.

RAYMOND, D. and Phaff, H.J. 1965. Purification and certain properties of avocado polygalacturonase. *J Food Sci* **30**, 266-273.

ROUSE, A.H. and Atkins, C.D. 1955. Pectin esterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experimental Station. *Fla Agric Exp Sta Bull* **570**, 1-19.

SOMOGYI, M. 1952. Notes on sugar fermentation. *J Biol Chem* **195**, 19-23.

TALMADGE, K.W., Keegstra, Bauer, W.D. and Albersheim, 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of pectic polysaccharides. *Plant Physiol* **51,** 158-173.

WALTON, J.D. 1994. Deconstructing the cell wall. *Plant Physiol*, **104**, 1113-1118.

ZAUBERMAN, G and Schiffmann-Nadel, M. 1972. Pectin methylesterase and polygalacturonase in avocado fruits at various stages of development. *Plant physiol* **49,** 864-865.