Pectin Methyl-*trans*-eliminase as the Maceration Factor of *Sclerotinia fructigena* and Its Significance in Brown Rot of Apple

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(Accepted for publication 11 January 1968)

SUMMARY

Good correlation between pectin methyl-*trans*-eliminase (PTE) activity and the ability to macerate plant tissue slices was found in fractions obtained from culture filtrates of the fungus *Sclerotinia fructigena* by gel filtration on dextran gel, or by ion-exchange chromatography. The presence of two isoenzymes was indicated, one of which was markedly activated by sodium polypectate or potato extract. Endo-polygalacturonase and α -L-arabinofuranosidase activities were not correlated with macerating ability. Negligible amounts of PTE were, however, detected in extracts of apple fruitlets rotted by *S. fructigena*; the pH optima of the enzyme components in the culture filtrates differed greatly from the pH of apple tissue. For this and other reasons PTE would appear to have little, and at most very localized, significance in the infection of apple fruits by *S. fructigena*.

INTRODUCTION

The fungus *Sclerotinia fructigena* Aderh. & Ruhl. causes a brown rot disease of apple fruits characterized by the comparative firmness of the invaded tissue, which is a result of the relatively small degradation of pectic components during fungal attack (Cole, 1956; Cole & Wood, 1961). Culture filtrates of this fungus are known to contain polygalacturonase (PG; Cole, 1956) and to be able to macerate plant tissue. It has been suggested that generally such maceration of plant tissue by fungal enzymes can be related to PG, with or without pectin methylesterase (Demain & Phaff, 1957), and that it is not necessary to postulate a specific 'protopectinase'. Bateman (1963) subsequently identified the macerating enzyme of *Rhizoctonia solani* as a polygalacturonase. However, by the use of gel filtration and cellulose chromatography it has been found possible to resolve the PG and macerating activities of a culture filtrate of *S. fructigena*, and to obtain preparations devoid of PG but still capable of tissue maceration (Byrde & Fielding, 1962). This action was attributed to a 'maceration factor', still biochemically undefined. Subsequently the presence of an enzyme capable of liberating L-arabinose from potato fibre was demonstrated in such macerating fractions and the enzyme was found to be an α -L-arabinofuranosidase (Byrde & Fielding, 1965).

Albersheim, Neukom & Deuel (1960) showed the existence of an enzyme which cleaved the polygalacturonate chain by an eliminative instead of by a hydrolytic mechanism. The role of enzymes of this general type (*trans*-eliminases) in tissue maceration has subsequently been shown in several instances (e.g. Bateman & Millar,

1966; Dean & Wood, 1967). The present paper identifies the 'maceration factor' of *Sclerotinia fructigena* as a pectin methyl-*trans*-eliminase, and not as an α -L-arabinofuranosidase. The possible significance of the former enzyme in the host/parasite relationship of brown rot of apple has also been examined.

METHODS

Organism. The culture of *Sclerotinia fructigena* used was initially isolated from a naturally infected apple, and stock cultures were maintained on potato + carrot agar (Dade, 1960). For conidial production, the fungus was grown in boiling tubes on slopes (20 ml.) of 15 % (v/v) 'V. 8' mixed vegetable juice (Campbell's Soups Ltd., King's Lynn, Norfolk), containing 3 % (w/v) agar. After 3 days at 25°, the cultures were transferred to daylight (but not direct sunlight) at room temperature to promote sporulation, which is light-induced in this species (Hall, 1933). Spores were normally taken from 7-day cultures.

Routine production of enzymes in vitro. The fungus was grown on the following medium (pH 5.7), similar to that used by Cole (1956), dispensed in 200 ml. lots in penicillin-culture flasks: sodium polypectate (Exchange Lemon Products Co., California), 10 g.; ammonium tartrate, 15 g.; KH_2PO_4 , 1 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; water to 1 l. After autoclaving, the medium was inoculated with a dense conidial suspension and the culture incubated for 11 days at 25°. The culture filtrate (about pH 7.0) was harvested by filtration, stored at 0° to -4° and concentrated by partial 'freezing out' (Dixon & Webb, 1958, p. 57).

Time-course of enzyme induction. For a more critical evaluation of enzyme induction the fungus was obtained in pellet form in liquid culture by inoculating a medium of 1.5 % (w/v) glucose and 3.5 % (w/v) malt extract (dispensed in 50 ml. lots in 250 ml. conical flasks) with a dense spore suspension of *Sclerotinia fructigena*. This medium, as used by McCallan & Miller (1957) for *S. fructicola*, was the only one tested in which satisfactory pellets could be grown. Flasks were incubated overnight at 25° without shaking (on the shaker the germinating spores were liable to clump at this stage). The following morning the flasks were transferred to a reciprocating shaker (100 strokes/min., stroke 4 cm.) at 25° and shaken for a further 30 hr before harvesting the mycelial pellets by centrifugation in sterile tubes and washing the mycelial deposit with sterile water.

The test system for induction was as follows: 0.5 ml. thrice-washed mycelial pellets, with 3.0 ml. sterile basal medium, in a 50 ml. flask. The basal medium comprised: malic acid, 10 g.; KH_2PO_4 , 2 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; NH_4Cl , 5 g.; water to 1 l.; adjusted to pH 4.0 with 2.5 N-NaOH.

After incubation overnight, with the object of depleting excess polysaccharide in the mycelial pellets (otherwise found to delay enzyme induction), the inducing substrates were added, usually as 1 ml. sterilized solution in basal medium. The cultures were replaced on the shaker for a further period before finally being harvested by filtration. The culture filtrates were retained for enzyme assay, while some of the mycelial pellets were inoculated into wounds in apple fruitlets, as described below.

Enzyme production in vivo. Fruitlets of apple var. Laxton's Superb were harvested in early July, and infected by wounding with a sterile scalpel followed by the insertion of a mycelial inoculum of *Sclerotinia fructigena*. For a comparison with enzyme in-

duction *in vitro* on a time basis, wounds were made with a special instrument resembling a cork borer, the depth of its cut being limited to 3 mm. by a flange; the disc cut (5 mm. in diam.) was then removed with a needle. The inoculum comprised mycelial pellets of *S. fructigena*, grown in liquid medium as described, and washed in sterile water. Lesion development was measured at intervals along two predetermined diameters at right-angles to each other.

Host tissue, both infected and healthy, was extracted with sodium chloride (5 g./100 ml. in water or in McIlvaine buffer, pH 8 (w/v)) or with 0.5 M-sodium carbonate containing 0.1 g. sodium dithionite/100 ml. at the rate of 10 ml. extractant to 5 g. host tissue. When sodium carbonate solution was used, the extract was mixed immediately after filtration with twice its volume of McIlvaine buffer (pH 6) to decrease the pH value and minimise enzyme inactivation.

Resolution of enzyme components. Gel filtration was done on Sephadex G 75 dextran gel (bead form), in a column approximately 80 × 4.0 cm. Concentrated culture filtrate (25 ml.) was applied, followed by elution with water. Fractions (10 ml.) were collected and their enzyme activities assayed.

For ion-exchange chromatography, CM-Sephadex (C-50) was used. This was taken up in 0.05 M-acetate buffer (pH 5.8), and to a column approximately 25 × 3.0 cm. was added 10 ml. concentrated culture filtrate which had been dialysed for 6 hr against distilled water and then overnight against the buffer. Gradient elution, achieved by using a mixing chamber and constant pressure by means of a Mariotte flask, was done with increasing concentrations of NaCl (from zero to 0.1 M in the acetate buffer); 10 ml. fractions were collected and assayed as before.

Enzyme estimations

Polygalacturonase (PG) was estimated by the cup-plate assay of Dingle, Reid & Solomons (1953) using sodium polypectate as substrate. The diameter of the white ring obtained on the agar plate was proportional to the logarithm of enzyme concentration over a range of dilutions. Activities were expressed relative to an aqueous solution (1 mg./ml.) of the commercial enzyme 'Pectinol 10 M' (Rohm & Haas Ltd.), defined arbitrarily as having 100 units activity/ml. The white rings are thought to be due to the presence of oligo-uronides insoluble at pH 1, which are intermediate products of hydrolytic degradation (Dingle *et al.* 1953). Whilst it is possible that the unsaturated products of a *trans*-eliminative breakdown might react similarly, this mechanism of degradation of polypectate by *Sclerotinia fructigena* preparations was discounted by means of u.v. spectrophotometry (Table 3).

α -L-Arabinofuranosidase (AF). The method used (see below) was based on the liberation of *p*-nitrophenol from the chromogenic substrate *p*-nitrophenyl α -L-arabinofuranoside, prepared by the method of Fielding & Hough (1965).

β -D-Galactopyranosidase (GP) was estimated by a similar method with *o*-nitrophenyl β -D-galactopyranoside (Koch-Light) as substrate (1.0 mg./ml.). The test system comprised: enzyme solution (diluted when desired), 1.0 ml.; 0.1 M-acetate buffer (pH 4.7), 5.0 ml. After equilibration at 30°, substrate solution (0.5 ml., 0.5 mg./ml.) was added, and the mixture incubated at 30°. The reaction was stopped by adding saturated Na₂CO₃ solution (1.0 ml.), which also developed the yellow colour of the *p*-nitrophenate ion. This was read on a Spekker absorptiometer with an Ilford no. 601 violet filter, against an appropriate substrate blank. Necessary enzyme blanks were

also included. One unit was defined as that amount of enzyme which catalysed the hydrolysis of 1 μ mole of substrate per min. at 30° (*Enzyme Nomenclature*, 1965).

The low substrate concentration (0.5 mg./ml.), which imposes limits on the AF assay, was used because it was in short supply.

Pectin methyl-trans-eliminase (PTE) was estimated by a method based on that of Albersheim & Killias (1962). A solution of pectin (2 g./100 ml.; Brown Ribbon brand, Union Crystalex Gelatine Ltd., London) was first centrifuged at 34,000 g to remove colloidal particles (Mr R. C. Codner, private communication) and one volume mixed with 3 volumes of McIlvaine buffer or 0.05 M-tris HCl buffer of appropriate pH value. To 4 ml. of this 0.5 % solution of pectin, equilibrated at 30°, was added 0.2 ml. of the test enzyme solution, and the extinction was read on a spectrophotometer immediately after mixing and again after an appropriate incubation time. Activity was expressed arbitrarily as the increase/100 min. in extinction at 240 m μ , the wavelength at which maximum absorption of the products of the enzyme reaction was found to occur (Fig. 1). Generally, a wavelength of 235 m μ has been used by other workers, but Cole (1967) also recorded maximum absorption at higher wavelengths under certain conditions, using the corresponding enzyme from *Penicillium digitatum*.

Macerating activity was estimated by following the loss of coherence at 25° of discs of potato tissue (Brown, 1915). Plugs 1 cm. diam. were cut from potato tubers, and immersed in water for 20 min. under reduced pressure. Discs 0.35 mm. thick were then cut by using a hand microtome, and sets of four discs transferred to 2 ml. test solution in watch-glasses. Samples of fractions to be tested were arranged in a random order. Maceration activity was expressed as 100/ t , where t was the time (min.) for the discs to lose coherence under the gentle pull of dissecting needles. One maceration test was done on apple tissue; discs were 0.40 mm. thick and the tissue did not receive the reduced pressure treatment.

Inactivation experiments. For temperature inactivation experiments, enzyme preparations were held at the appropriate temperature for 20 min. before being cooled rapidly by immersing the tubes in ice-water.

For pH inactivation, 1 ml. of a suitable gel-filtration fraction free from low molecular weight material was added to 4 ml. citrate phosphate borate buffer (of required pH; Teorell & Stenhagen, 1938) and the pH value of the mixture recorded. After 20 min. at room temperature 0.2 ml. was added to 2 ml. McIlvaine citrate phosphate buffer (pH 5.2) for pectin methyl-trans-eliminase assay following the addition of pectin (1.0 %, 2 ml.). For assay of macerating activity 1 ml. of the first-named mixture was added to 4 ml. McIlvaine buffer (pH 5.2) and 2 ml. of this mixture used for assay.

RESULTS

Concentrated culture filtrate (25 ml.) was partially resolved by gel filtration through Sephadex G 75, and fractions assayed for the enzymes PG, AF and PTE and maceration of potato discs (at pH 5.2 for PTE estimation). The elution pattern took the form of two peaks. Figure 2 shows the relationship between PTE and macerating activity for the fractions tested (every third one): the partial correlation coefficient for these data was +0.883 ($P < 0.001$). By contrast, the partial correlation coefficient between PG and macerating activity was +0.271 (not significant), and between AF and macerating activity -0.190 (not significant). A similar relationship was evident in

seven other gel-filtration experiments, though no statistical correlation was attempted. On no occasion were anomalous results obtained.

A dialysed concentrated culture filtrate (pH 6.5, 10 ml.) was applied to a column of CM-Sephadex which was then eluted, and fractions (10 ml.) tested for PG, AF, PTE and macerating activity on potato and apple discs (Fig. 3, 4). Figure 4 also shows that the two PTE components differed in their pH/activity relationships. However, even taking pH effects into account, the macerating activity of the second component

Fig. 1

Fig. 2

Fig. 1. Ultraviolet absorption of products of pectin methyl-*trans*-eliminase action. Test 1: pH 8.0 in tris HCl buffer; pectin concentration 0.5 %; enzyme from dialysed culture filtrate concentrate; incubation time, 30 min. Test 2: pH 5.2 in half-strength McIlvaine buffer; pectin concentration 0.25 %; enzyme from gel filtration fractions; incubation time, 100 min.

Fig. 2. Relationship between macerating activity and pectin methyl-*trans*-eliminase (at pH 5.2) in fractions from gel filtration.

was greater than that expected on the basis of its PTE activity. This result could however be explained if the second component were activated in the maceration assay. In a subsequent experiment the effect of an aqueous extract of potato tuber (both dialysed and undialysed) and of Ca^{2+} , Mg^{2+} and K^{+} on the PTE activity of the second component was therefore examined. Activation by both potato preparations was observed, but the dialysate from the potato extract, and also the inorganic cations, had negligible effect. Table 1 shows that only the second PTE component was thus activated. Similar results were obtained in subsequent tests when an extract of cucumber fruit was substituted for potato extract. Table 1 shows that similar activation could also be effected by a boiled culture filtrate of *Sclerotinia fructigena* grown on the usual polypectate medium, or by an aqueous solution of sodium polypectate, itself subject to negligible PTE attack, or to a lesser degree, by polygalacturonic acid.

In a subsequent fractionation with CM-Sephadex, sodium polypectate was added to the assay mixture for PTE for each fraction tested, at pH 6.0 (about that of the potato tissue). The pattern of the results obtained (Fig. 5, 6) shows the much closer relationship between PTE and macerating activity than in the previous fractionation when sodium polypectate was not included in the assay. Figure 5 also shows the presence of a β -galactosidase at the second PTE peak.

The pH/activity relationships of the two PTE isoenzymes were examined in the presence of sodium polypectate, with the results shown in Fig. 7.

Fig. 3

Fig. 4

Fig. 3. Polygalacturonase (●), α -L-arabinofuranosidase (■) and macerating (○) activities of fractions from gradient elution of CM-Sephadex. Maceration of apple discs (not shown) was maximal at fractions 14 and 28 (activity 0.75).

Fig. 4. Pectin methyl-*trans*-eliminase activities of CM-Sephadex fractions corresponding to Fig. 3 at pH 6.0 (■), 7.0 (○) and 8.0 (●).

Table 1. Activation of pectin methyl-*trans*-eliminase components from *Sclerotinia fructigena* culture filtrate obtained by CM-Sephadex fractionation

Test	Activator*	pH value of assay	$\Delta E_{240}/100 \text{ min.}$	
			First PTE component	Second PTE component
1	Potato extract	5.3	0.029	0.246
	Water	5.3	0.008	0.023
2	Boiled culture filtrate	5.3	—	0.113
		8.0	—	0.155
	Sodium polypectate (2 %)	5.3	—	0.093
		8.0	—	0.132
3	Water	5.3	—	0.000
		8.0	—	0.060
	Sodium polypectate (2 %)	8.0	—	0.133
	Polygalacturonic acid (2 %)	8.0	—	0.064
	Water	8.0	—	0.048

* 0.2 ml. added to 4.2 ml. of usual assay system. Appropriate activator/enzyme blanks were included and gave readings less than 0.02.

— signifies no test.

Effects of exposure to high temperature and extreme pH values

From plots of residual activity against temperature of exposure (20 min.) in three experiments the following mean values for 50 % decrease of activity were obtained: macerating activity, 49.4°; PTE, 48.0°.

Fig. 5

Fig. 6

Fig. 5. Polygalacturonase (○), α-L-arabinofuranosidase (●) and β-galactopyranosidase (□) activities of fractions from gradient elution of CM-Sephadex.

Fig. 6. Macerating activity (●) and pectin methyl-*trans*-eliminase activity in the presence of sodium polypectate at pH 6 (○) of fractions corresponding to Fig. 5.

Fig. 7

Fig. 8

Fig. 7. pH/activity relationship for pectin methyl-*trans*-eliminase isoenzymes in fractions 24 (○) and 48 (●).

Fig. 8. Residual pectin methyl-*trans*-eliminase (●) and macerating (○) activities following exposure (20 min.) to different pH values.

The effect of exposure (20 min.) to different pH values is shown in Fig. 8, which shows the comparatively high, and similar, tolerance of both activities towards extremes of pH.

Extraction of host tissue

Polygalacturonase was readily detected in all extracts of apple fruitlets freshly rotted by *Sclerotinia fructigena*, but not in healthy tissue (see Akinrefon, 1967). Repeated attempts to detect quantities of PTE above the value of experimental error were unsuccessful, at pH 4, 6 or 8. However, the extract of rotted tissue showed no inhibitory effect on PTE when mixed with a gel filtration fraction of high activity.

Table 2. *Polygalacturonase and pectin methyl-trans-eliminase in filtrates of young Sclerotinia fructigena cultures*

Test no.	Substrate	Final conc. in basal medium (% w/v)	PG (u.).		PTE*.	
			Incubation time		Incubation time	
			8 hr	24 hr	8 hr	24 hr
1	Pectin	1.0	15.8	—	0.04	—
	Apple fibre†	1.0	8.0	—	0.01	—
	Nil	—	4.0	—	0.04	—
2	Apple fibre†	0.5	6.3	25.1	0.00	0.05
	Nil	—	1.3	2.3	0.01	0.01
Comparable figures for routine 11-day cultures...			400		4.13 (pH 8)	

* Assayed at pH 5 in test 1; at pH 8 in test 2 ($\Delta E_{240}/100$ min.).

† Prepared by the method of Cole & Wood (1961); not sterilized.

— signifies no test.

Induction of pectolytic enzymes in vitro

Table 2 summarizes the results of two experiments in which mycelial pellets of *Sclerotinia fructigena* were exposed to different substrates. After harvesting, the culture filtrates were assayed for enzyme activities. Similar pellets harvested 6 hr after the substrates were added were used as inoculum for standard wounds (5 mm. diam.) made in apple fruitlets. Rots developed rapidly, reaching mean diameters of 9 mm. and 13.5 mm. after 16 and 23 hr respectively. There were statistically significant differences in rot diameter following different induction treatments: mycelial pellets from apple juice gave the largest diameters. Control wounds, which received no inoculum, did not become infected.

Enzymes not involved in maceration

During experiments on the biochemical identity of the 'maceration factor', a number of hypotheses were tested and subsequently discarded: these are summarized in Table 3.

DISCUSSION

The results presented identify the 'maceration factor' in culture filtrates of *Sclerotinia fructigena* as a pectin methyl-trans-eliminase (PTE) enzyme. The gel filtration pattern showed a highly significant correlation between these two activities, but no correlation between rate of maceration and the activities of polygalacturonase and α -L-arabinofuranosidase. In earlier work, preparations with macerating activity but devoid of polygalacturonase were obtained (Byrde & Fielding, 1962); purified polygalacturonase preparations have subsequently been found to have negligible macerat-

Table 3. *Hypotheses for action of 'maceration factor' which were subsequently discarded*

Chemical group or linkage involved	Reference to role in plant structure	Substrate used in tests	Assay of incubation mixture (normally pH 5)	Result*
Cellulose	Wood (1960)	(a) Cellulose	Paper chromatography	A
		(b) Carboxymethyl-cellulose	Viscometric estimation	A
Protein	Ginzburg (1961)	(a) Gelatin-charcoal discs	Charcoal liberation (Kohn, 1953)	A
		(b) Potato fibre	Absorption at 280 m μ wavelength	A
Hydroxyproline-rich protein	Lamport (1965)	Hydroxyprolylglycine and glycyhydroxyproline	Paper chromatography and hydroxyproline assay	A
Xylan	Sørensen (1957), Hancock & Millar (1965)	(a) Xylan	Paper chromatography	A
		(b) <i>o</i> -Nitrophenyl- β -D-xyloside (Fisher <i>et al.</i> 1966)†	Colorimetric estimation	A
Methoxyl groups in pectin	Joslyn (1962)	High-methoxyl pectin	Methanol estimation (pectin methylesterase assay)	B
Glycosidic linkages in polygalacturonate chain	Wood (1960)	Sodium polypectate	Absorption at 230 m μ wavelength (polygalacturonate <i>trans</i> -eliminase assay)	A
Aldobiuronic acid units in polygalacturonate chain	Barrett & Northcote (1965)	Pectinic acid	Column chromatography of partial acid hydrolysate (Barrett & Northcote, 1965, fig. 7)	A
Phosphate ester linkages between pectin chains	Henglein (1958)	(a) Potato fibre or pectin	Inorganic phosphate estimation	A
		(b) Bis (<i>p</i> -nitrophenyl) phosphoric acid, Na salt	Colorimetric estimation	A
Calcium and other divalent ions as 'bridges' between pectin chains	Joslyn (1962), Bateman & Millar (1966)	(a) Potato fibre	Calcium estimation	A
		(b) Potato discs	Effect of 0.1 M-Ca (NO ₃) ₂ and 0.01 M-EDTA on rate of maceration	A

* A, no activity in crude culture filtrate; B, culture filtrate active but no activity in purified 'maceration factor' preparations.

† Kindly supplied by Dr P. W. Kent.

ing activity (Byrde & Fielding, unpublished). A similar close relationship was apparent in the elution pattern from a CM-Sephadex ion-exchange dextran column, but only when PTE was assayed at pH 6 in the presence of sodium polypectate (in addition to the pectin substrate). The results showed the unexpected existence of two PTE isoenzymes: that which eluted first had an activity optimum at pH 8.3, and was not activated by potato extract. The second isoenzyme had an activity optimum at pH 7.3 and was strongly activated by potato extract or sodium polypectate. Account should thus be taken of such activation effects when assessing the role of purified enzyme preparations in tissue maceration: it is likely that the activator known to be present in the crude culture filtrate became separated from the enzyme during the CM-Sephadex fractionation.

The pH optima recorded for these isoenzymes are considerably higher than those

quoted for other pectin methyl-*trans*-eliminase enzymes (as distinct from polygalacturonate *trans*-eliminases), which are generally about pH 5 (see e.g. Albersheim & Killias, 1962), although a second peak at pH 8.5 in the presence of Ca^{2+} was reported by Edstrom & Phaff (1964). Further biochemical investigation of the enzyme, such as the mechanism of its activation and whether it attacks by a random or terminal mechanism, is clearly required.

The effects of pH value on the stability of the maceration factor and of PTE also showed good agreement, and the slight discrepancy between the temperature inactivation data may be due to departures from linearity in the use of an inverse time function as a measure of maceration activity (see McClendon & Somers, 1960; Cole, 1967).

The results summarized in Table 3 show that a number of other enzymes, which include polygalacturonate *trans*-eliminase, could not be identified with tissue maceration by *Sclerotinia fructigena*. The absence of cellulase activity is probably a factor in the long survival of mummified fruits following infection: in any event, cellulases do not generally appear to play a part in maceration (Bateman & Millar, 1966).

The pH optima for both the PTE components are considerably higher than those normally encountered by *Sclerotinia fructigena* in host tissue. The pattern of maceration of potato shown in Fig. 6 appears to be the expression of PTE activity at the pH value of the tissue (6.5) and the same is probably true of apple fruitlet tissue, which has a lower pH value (3.5) and macerated more slowly than did potato tissue. This raises the question of the significance of the PTE enzyme in the brown rot disease of apples, where the host may be lower than pH 3.0. In addition, two other results cast doubt on the role of the enzyme. First, negligible amounts of PTE activity were recovered from infected tissue, and this lack of enzyme activity did not appear to be due solely to inactivation. Similarly, Cole & Wood (1961) detected no macerating activity in extracts of apple tissue rotted by *S. fructigena*. However, appreciable quantities of polygalacturonase can be extracted, particularly from the oldest part of the rot (Akinrefon, 1967). Secondly, negligible amounts of PTE were secreted by mycelial pellets of *S. fructigena* during the first 24 hr of incubation *in vitro* under conditions in which polygalacturonase was rapidly formed. These pellets were, however, capable of rapid invasion of host tissue within 16 hr. In the invasion of apple tissue the possibility of the local secretion of a small, but temporarily effective, quantity of enzyme, which is subsequently inactivated cannot be finally excluded (see Cole & Wood, 1961). However, the weight of evidence indicates that the genetic ability of *S. fructigena* to secrete pectin methyl-*trans*-eliminase is not utilized, and the relatively firm nature of the resultant rot is in accordance with this view.

We thank Miss Celia Slowley for valuable technical assistance, Miss M. Holgate for the correlation analysis and Mr E. G. Bradfield for calcium estimations.

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