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EFFECT OF AGE AND STORAGE CONDITIONS ON THE SUSCEPTIBILITY OF CERTAIN VEGETABLES TO ATTACK BY TISSUE-ROTTING FUNGI.

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(With 3 Text-figures)

Abstract

This investigation has been undertaken to study the basis of resistance shown by the average host to the attack of the average fungus, exemplified by the behaviour of *Botrytis cinerea* to living potato tissue. For this purpose, susceptibility of certain vegetable tissues, brought to a definite physiological condition, to the attack of a number of tissue-rotting fungi has been studied.

Pre-storage at 30°C. or 35°C. (as compared with 15°C.) increases the susceptibility of certain plant tissues (potato, swede, turnip, carrot) to invasion by a number of fungi, whether normally parasitic upon these hosts or not. The effect increases with length of the pre-storage period, and is greater at 35°C. than at 30°C.

Susceptibility of potato tubers varies with their maturity. Young tubers are susceptible to attack by certain fungi which are classed as non-pathogenic to potato; as the tubers mature, susceptibility decreases and reaches a very low value; at the time of dormancy it then increases with sprouting. With *Pythium de baryanum* and *Phytophthora erythroseptica* which are normal parasites of potato, susceptibility is low with young tubers and increases continuously with advancing maturity and finally with sprouting. When dormant tubers are caused to sprout by artificial means, susceptibility is increased.

Heat pre-treatment beyond a certain time has a damaging effect on tissue, as determined by the plasmolytic method. The safety limits were determined for the tissues used. Heat pre-treatment increases the absorbing capacity of the cells, has no effect on *p*H of the cell-sap and brings about change in the cell-wall substance which are reflected in its staining reactions and in the greater readiness with which it is decomposed by fungal enzymes. Heat pre-treatment of tissue increases the exosmosis of salts and sugars from the cells. This result was demonstrated by physical and chemical methods as well as by the effect of the diffusates in stimulating fungal germinations, growth and enzyme secretion.

The effect of maturity on susceptibility is not due to cell-wall changes but is closely correlated with exosmosis of nutrients.

The specificity of some of the fungi used to tissues is due in part at least to reactions arising from the nature of the plant sap. These may interfere with growth of the fungus, with secretion of the pectinase enzyme or with the functioning of the latter. Illustrations of these effects are given.

The amount of rotting brought about by a small quantity of fungal enzyme, when applied to a relatively large piece of potato tissue, is not correlated with the readiness with which the enzymic liquid is absorbed by the sub-turgid tissue. It is determined by the concentration of the enzyme present, and by the source of the latter. *Botrytis* enzyme is less effective than *Pythium* enzyme of the same strength, as determined by the time required to macerate discs of tissue of standard thickness.

Introduction

The nature of host resistance to the attack of a parasite has always been a subject of interest in plant pathology, either from the academic

or the practical point of view. The internal resistance offered by the host to the attack of an invading organism generally falls into two categories—mechanical and chemical. The former is concerned either with the various properties of the cell-wall e.g., structure, composition, thickness, or the capacity of the cell to develop some barrier in the form of cork cells or gum in advance of the pathogen. The chemical factors that are responsible are usually the acidity of the cell-sap or the presence of certain toxic substances in the cell such as tannins or glucosides.

As pointed out by Brown (1934, 1936) a more general and widely occurring type of resistance is that shown by the average host to the attack of the average fungus. In such cases, the host tissue may have nothing inhibitory or harmful to the growth of the fungus and the fungus also may not require any special food for its growth or enzyme secretion. Nevertheless, attack does not occur even though the necessary conditions seem to be present. A familiar example of this is the behaviour of *Botrytis cinerea* in relation to living potato tissue. *Botrytis cinerea* can grow well on potato decoctions and on dead potato tissue, and is capable of secreting the enzyme pectinase under those conditions, but under normal conditions there is no attack.

The present investigation has been undertaken with a view to determine more closely the basis of resistance of the host tissue in such cases of general nature. Certain vegetable tissues have been chosen for this work, as being readily available and amenable to experimental treatments. After certain treatments which were devised so as to bring these tissues into a certain physiological condition, they were tested in a systematic manner with regard to their resistance to invasion by certain fungi. With such quantitative data available, it was hoped that a closer insight would be obtained into the mechanism of resistance on the one hand and the nature of the aggressive apparatus of the fungus on the other.

Historical

Effect of storage at high temperature prior to inoculation

Vasudeva (1930) first observed that apples of the Newton variety, when artificially "ripened" by storage at 30°C. for several days, were attacked by *Botrytis allii* while the control ones kept at laboratory temperature were not. He determined the acidity, sugar and nitrogen contents of the extract of apples after storage at the temperatures stated but was unable to explain the changed susceptibility to attack on the basis of changes in any of these factors.

A similar result was obtained by Chona (1932) who noted that apples of the Newton variety after storage at 35°C. for 7 days became more susceptible to attack of two strains of *Fusarium fructigenum*.

Later, Fernando (1934) found that *Bacillus subtilis*, which is normally a saprophyte, can attack potato tubers of the Epicure variety if they are stored previously at 38–40°C. for several days.

Effect of Age

In apple, Horne (1930), Vasudeva (1930) and Chona (1932) found diminishing resistance to attack with gradual ripening. Horne (1930) considered that this was due to progressive diminution of the acidity of the cell-sap and to increase in the soluble nitrogen content with advancing age.

Chona (1932) found that Kerr's Pink variety of potato become more susceptible to the attack of *Fusarium caeruleum* with progressing age and maturity. This increase in susceptibility to attack with maturity had been observed by Pethybridge and Lafferty (1927) and later by Small (1944). Small (l.c.) also observed that young immature tubers are usually not susceptible to the attack of *F. caeruleum* but that there were exceptions to this rule.

Material and methods

Fungi and host tissues used

In the present investigation, the following fungi were used :—(1) three different strains of *Botrytis cinerea* Pers., namely, (*a*) *Lettuce strain* (from stem lesion), (*b*) *Daffodil strain* (from diseased bulb) and (*c*) *Gladiolus strain* (from diseased corm); (2) *Fusarium fructigenum* Fries. ("A" strain of Brown—from apple fruit); (3) *Rhizopus nigricans* Ehr.; (4) *Pythium de baryanum* Hesse (from a rotted potato); (5) *Phytophthora erythroseptica* Peth. (from diseased potato tuber) and (6) Two different strains of *Rhizoctonia solani* Kuhn., namely, (*a*) Potato strain (from diseased sprout) and (*b*) Crucifer strain (from diseased seakale leaf).

All these fungi were obtained from stock cultures kept at the Plant Pathology Laboratory, Imperial College of Science and Technology. The cultures of *Fusarium fructigenum*, *Rhizopus nigricans* and the three strains of *Botrytis cinerea* were maintained on slopes of potato agar, the two strains of *Rhizoctonia solani* on malt agar and *Pythium de baryanum* and *Phytophthora erythroseptica* on oatmeal agar.

In those experiments where only one strain of *Botrytis cinerea* was used, the lettuce strain was always chosen.

Two varieties of potato tubers, namely King Edward and Majestic, were chiefly used in this work. The other varieties used from time to time were Epicure, Sharpe's Express, Arran Pilot, Great Scot, Arran Banner, and Up-to-date. The other vegetables studied were swede and carrot.

Method of inoculation

As a routine measure, spores required for inoculations were taken from 10–15 days old cultures and mycelial pieces from 4–6 days old cultures. To eliminate contaminants, potato tubers and swede roots were first washed in water, then dipped for 10 minutes in 0.2% solution of mercuric chloride and finally washed with water. Potato tubers and root vegetables were inoculated by introducing the fungus into the host tissue after the method of Granger and Horne (1924). This was standardised by inserting the cork borer (No. 2 size) to a definite depth (1.5 cm.). After inoculation,

the vegetables were stored in large sterile tin boxes. There was no necessity for any arrangement to maintain humidity inside the box as the water lost by the vegetables during storage was sufficient to maintain a moist atmosphere inside the box. In all cases, the inoculated material was incubated at 20°C. With potato tubers and root vegetables, the amount of attack was determined by weighing the quantity of rotted tissue after 7 days inoculation. In each determination of the amount of attack, ten replicates were always used.

Preparation of pectinase

To obtain the enzymic preparation of *Botrytis cinerea*, Brown's plate technique (1915) was employed. The dried mycelium after being powdered with an equal quantity of sand, was used for obtaining the enzyme. A watery suspension of enzyme was prepared by digesting the powder with water, the usual quantity being 0.2 gm. of powder in 3 c.c. of water.

To prepare the enzyme of *Pythium de baryanum*, Menon's (1934) method was mainly used.

An alternative method which gave a stronger enzymic solution from *Pythium de baryanum* was to grow the fungus in a flask containing 10 c.c. of the following medium : Asparagin 4.5 gm., Glucose 5.0 gm., Peptone 4.5 gm., $MgSO_4$ 1.6 gm., K_3PO_4 3.3 gm., Starch 25.0 gm. and Water 1,000 c.c. After 10 days' growth at 20°C., the liquid was used as such as a source of enzyme.

Determination of enzymic activity

This was done according to Brown's method (1915) by noting the time taken for the loss of coherence of discs of potato (1.5 cm. in diameter and 0.5 mm. in thickness) when they are immersed in enzymic solutions. In such tests, 3 discs were placed in 3 c.c. of the liquid.

Effect of pre-storage temperature on amount of attack

(a) *Potato*

The main experiments under this heading were carried out with a large batch of tubers of the varieties King Edward and Majestic. In order to secure as much uniformity as possible, tubers of approximately equal size were sorted out, and half of each batch stored at 15°C, and the other half at 35°C. At intervals, tubers were taken out, inoculated with the test fungi and then stored at 20°C. At the end of 7 days incubation (at 20°C.), the amount of rotted tissue was weighed in each case. For each determination of rotting due to each fungus, 10 tubers were used. Each figure in the Tables (1–2) thus represents the mean of 10 determinations. This series of experiments was carried out from January to early March, i.e., at the period when the tubers are dormant and unsprouted (in England). Of the fungi used, only three, viz., *Pythium de baryanum*, *Phytophthora erythroseptica* and *Rhizoctonia solani* (potato) are normally associated with potato and thus are able to produce a material amount of rotting

Table 1. *Effect of pre-storage at 35°C. and 15°C on susceptibility of* King Edward potato

Temp. of pre-storage	Period of storage (days)	*Botrytis cinerea* (lettuce)	*Botrytis cinerea* (daffodil)	*Botrytis cinerea* (gladiolus)	*Fusarium fructigeu*	*Rhizopus nigricans*	*Pythium de baryanum*	*Phytophthora eryroseptica*	*Rhizoctonia solani* (potato)	*Rhizoctonia solani* (crucifer)
	1	0.027± 0.015	0.021± 0.012	0.069± 0.017	0.164± 0.014	0.084± 0.034	0.462± 0.057	3.380± 0.33	0.566± 0.066	0.072± 0.022
	2	0.148± 0.029	0.118± 0.023	0.305± 0.048	0.209± 0.042	0.134± 0.036	0.896± 0.046	4.574± 0.411	0.655± 0.016	0.078± 0.014
35°C	3	0.256± 0.022	0.266± 0.031	0.442± 0.038	0.388± 0.03	0.286± 0.01	1.195± 0.03	5.550± 0.34	0.733± 0.12	0.153± 0.024
	5	0.541± 0.053	0.355± 0.015	0.501± 0.033	0.415± 0.026	0.406± 0.028	1.316± 0.12	7.846± 0.48	0.915± 0.13	0.320± 0.019
	7	0.563± 0.025	0.465± 0.036	0.582± 0.027	0.555± 0.04	0.472± 0.035	2.121± 0.28	7.919± 0.51	1.073± 0.12	0.366± 0.021
	10	0.565± 0.035	0.475± 0.035	0.606± 0.026	0.713± 0.033	0.542± 0.032	2.634± 0.19	8.70± 1.33	1.10± 0.13	0.402± 0.012
	15	0.983± 0.075	0.881± 0.084	0.931± 0.083	0.887± 0.073	0.800± 0.057	7.312± 0.142	11.54± 1.49	1.481± 0.13	0.535± 0.022
	1	0.026± 0.012	0.022± 0.012	0.065± 0.023	0.154± 0.02	0.074± 0.02	0.463± 0.024	3.380± 0.33	0.522± 0.07	0.066± 0.027
	2	0.025± 0.009	0.021± 0.008	0.062± 0.017	0.148± 0.012	0.060± 0.036	0.484± 0.028	3.320± 0.41	0.520± 0.025	0.070± 0.023
15°C	3	0.022± 0.007	0.025± 0.008	0.058± 0.019	0.162± 0.034	0.068± 0.021	0.500± 0.059	3.150± 0.32	0.511± 0.033	0.070± 0.019
	5	0.032± 0.014	0.023± 0.007	0.056± 0.007	0.158± 0.028	0.071± 0.018	0.520± 0.036	3.168± 0.28	0.522± 0.038	0.072± 0.012
	7	0.030± 0.015	0.022± 0.005	0.051± 0.009	0.176± 0.031	0.066± 0.025	0.514± 0.073	3.364± 0.49	0.518± 0.039	0.080± 0.032
	10	0.031± 0.009	0.022± 0.008	0.056± 0.01	0.170± 0.029	0.076± 0.013	0.501± 0.041	3.170± 0.51	0.524± 0.063	0.084± 0.012
	15	0.026± 0.009	0.023± 0.008	0.060± 0.007	0.163± 0.025	0.070± 0.020	0.480± 0.075	3.522± 0.44	0.519± 0.039	0.071± 0.012

Table 2. *Effect of pre-storage at 35°C. and 15°C. on susceptibility of* Majestic potato

Temp. of pre-storage	Period of storage (days)	*B. cinerea* (lettuce)	*B. cinerea* (daffodil)	*B. cinerea* (gladiolus)	*F. fructigenum*	*R. nigricans*	*P. de barganum*	*P. erythroseptica*	*R. solani* (potato)	*R. solani* (crucifer)
35°C	1	0.084± 0.022	0.056± 0.02	0.133± 0.028	0.221± 0.024	0.128± 0.02	0.566± 0.072	3.50± 0.59	0.520± 0.01	0.081± 0.028
	2	0.211± 0.023	0.168± 0.025	0.211± 0.033	0.335± 0.018	0.260± 0.032	0.924± 0.062	4.348± 0.18	0.634± 0.024	0.139± 0.015
	3	0.519± 0.067	0.531± 0.059	0.533± 0.06	0.432± 0.012	0.298± 0.63	0.967± 0.092	5.931± 0.20	0.680± 0.034	0.208± 0.042
	5	0.526± 0.056	0.547± 0.043	0.616± 0.046	0.619± 0.02	0.478± 0.028	1.563± 0.296	8.120± 1.28	0.734± 0.029	0.236± 0.041
	7	0.546± 0.061	0.547± 0.033	0.632± 0.014	0.750± 0.04	0.590± 0.026	1.620± 0.18	9.293± 1.12	1.105± 0.06	0.346± 0.027
	10	0.652± 0.051	0.555± 0.029	0.671± 0.073	0.827± 0.03	0.622± 0.09	2.013± 0.31	10.405± 1.05	1.173± 0.101	0.398± 0.054
	15	1.353± 0.14	1.161± 0.22	1.553± 0.233	0.950± 0.048	0.903± 0.033	8.231± 0.88	14.260± 2.011	1.570± 0.11	0.521± 0.023
15°C	1	0.078± 0.029	0.065± 0.016	0.114± 0.02	0.196± 0.01	0.114± 0.028	0.562± 0.06	3.390± 0.43	0.501± 0.044	0.080± 0.023
	2	0.092± 0.022	0.069± 0.016	0.112± 0.026	0.214± 0.013	0.125± 0.013	0.560± 0.037	3.714± 0.64	0.518± 0.032	0.074± 0.036
	3	0.101± 0.018	0.074± 0.011	0.126± 0.017	0.026± 0.12	0.110± 0.011	0.582± 0.038	3.630± 0.57	0.510± 0.032	0.071± 0.013
	5	0.070± 0.014	0.070± 0.014	0.120± 0.031	0.226± 0.025	0.112± 0.033	0.548± 0.072	3.590± 0.27	0.524± 0.071	0.086± 0.02
	7	0.085± 0.017	0.080± 0.019	0.122± 0.028	0.214± 0.02	0.110± 0.019	0.522± 0.041	3.384± 0.40	0.499± 0.025	0.081± 0.027
	10	0.097± 0.023	0.083± 0.021	0.137± 0.023	0.206± 0.023	0.128± 0.025	0.582± 0.053	3.756± 0.31	0.520± 0.04	0.073± 0.017
	15	0.081± 0.029	0.068± 0.018	0.123± 0.019	0.199± 0.014	0.126± 0.024	0.567± 0.037	3.413± 0.26	0.523± 0.05	0.072± 0.009

under ordinary circumstances. The remainder are normally not parasitic upon potato tubers. In the case of *Botrytis cinerea* (3 strains) and *Fusarium fructigenum*, inoculations were made as uniformly as possible by adding 0.1 c.c. of a spore suspension; with others equal sized pieces of mycelium were used. The various particulars of the experiments are set out in the headings to Tables 1–2. The figures in these Tables give the mean amount (grams) of rotted tissue together with the standard error of each mean.

The analysis of variance has been worked out to show the significance of the effect of various factors, namely, variety, fungus, temperature, period and their interactions. The analysis has been carried out by substituting for each original figure (x) the quantity $\log_{10}$ 100x. The reason is that the weight of rotted tissue is a function of the amount of fungal growth which increases logarithmically. Under such circumstances, i.e., logarithmic growth, the variance increases with the square of the mean. In order to obtain a normally distributed error, it is therefore necessary to transform the data to a logarithmic scale. Another reason for this procedure is that the difference between smallest and largest figures in the Tables is very great, viz., 500 times. In such cases logarithmic transformation is necessary to bring the figures within comparable limits. The use of the logarithmic scale as pointed out by Fisher and Mackenzie (1923) and Cochran (1938) has also the advantage that it provides a better test of independence or interaction of treatments.

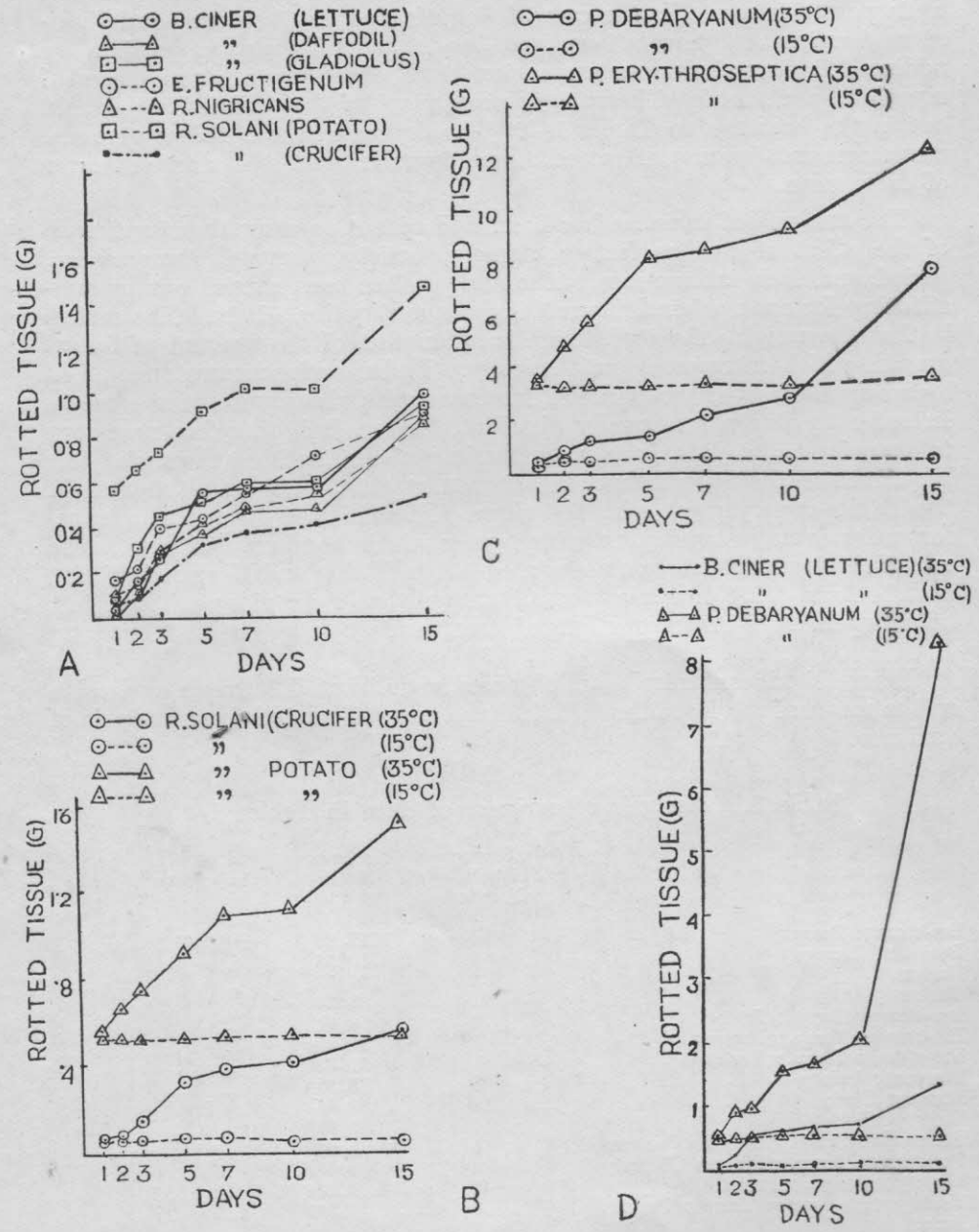
Where significant results are obtained, significant differences applicable to the mean of the logarithmic data have been calculated from analysis of variance.

Table 3. *Analysis of variance* of data in Tables 1–2

Due to	Degrees of Freedom	Variance Ratio (F)	Probablity
Pre-storage Temp.	1	1617.9	$P<0.01$
Period of Storage	6	10.54	,, ,,
Fungus	8	701.89	,, ,,
Variety	1	99.99	,, ,,
Pre-storage Temp. × Period	6	73.61	,, ,,
Pre-storage Temp. × Fungus	8	31.62	,, ,,
Pre-storage Temp. × Variety	1	21.83	,, ,,
Fungus × Variety	8	13.38	,, ,,
Fungus × Period	48	1.44	Not Sig.
Variety × Period	6	0.99	,, ,,
Pre-storage Temp. × Period × Fungus	48	1.00	,, ,,
Pre-storage Temp. × Fungus × Variety	8	0.48	,, ,,
Variety × Fungus × Period	48	0.184	,, ,,
Variety × Pre-storage Temp. × Period	6	0.041	,, ,,
Error	48		

The general results indicated in the Tables 1–2 and the statistical analysis of these may be summarised as follows :—

Pre-storage at 35°C. increases susceptibility of the tubers of the two varieties tested to rotting by the fungi studied. The susceptibility increases



Text-fig. 1. A, Effect of length of pre-storage at 35°C on amount of rotted tissue produced by different fungi on king Edward potato. B, Effect of length of prestorage at 35°C and 15°C on amount of rotted tissue produced by *P. de baryanum* and *P. erythroseptica* on King Edward potato. C, as in B by two strains of *R. solani* (potato and crucifer). D, Effect of length of pre-storage at 35°C and 15°C on amount of rotted tissue produced by *B, cinerea* and *P. de barynum* on Majestic potato.

with the duration of the pre-storage period. Tubers kept at 15°C. show practically the same susceptibility at all stages of the experiment and the constancy of the amount of attack shown on these by each fungus indicates that there was no change in the parasitic vigour of each fungus, as taken from various subcultures during the course of the experiment. The results given by the tubers stored at 15°C. constitute in fact the controls to those which were kept at 35°C.

The increased susceptibility arising from pre-storage at 35°C. is significant within 3 days or less for all the fungi except the two strains of *Rhizoctonia solani*. With these, difference becomes significant after 5 days. From the beginning of the pre-storage period until about the 5th day, increase of susceptibility is roughly proportional to duration of storage; from the 5th day to the 10th day, the rate of increase of susceptibility with time of storage is more gradual, but is followed by a rapid increase from the 10th day to the 15th day.

The fungi differ from each other in the absolute amount of rotting produced, but the same general tendency to increased attack on tubers pre-stored at 35°C. is shown by all. *Botrytis cinerea* (3 strains), *Fusarium fructigenum*, *Rhizopus nigricans*, and *Rhizoctonia solani* (crucifer) which behave as weak parasites under normal conditions, produce definite and substantial rotting when the tubers are kept previously at 35°C. for some time. In the case of *Pythium de baryanum*, *Phytophthora erythroseptica* and *Rhizoctonia solani* (potato), the attack is much augmented when the tubers are stored at 35°C.

There is a significant difference between the two varieties in the amount of attack under comparable conditions, Majestic being the more susceptible, both for tubers stored at 15°C. and at 35°C. This result applies, in particular, to the five fungi namely, *Botrytis cinerea* (3 strains), *Fusarium fructigenum* and *Rhizopus nigricans*. The greater susceptibility of Majestic shown in the experiments in Tables 1–2 was not always in evidence in comparisons done at other times. The effect shown in this case is therefore probably an accidental one, depending perhaps on the stock of tubers used at different times.

(b) *Swede*

Swedes were stored at 35°C., 30°C. and 15°C., and batches of 10 inoculated after the manner already described for potato. With this vegetable, the storage period at 35°C. could not be extended beyond 7 days on account of damage to the tissue arising from longer exposures. The experiment was conducted with four fungi, viz., *Botrytis cinerea* (Lettuce), *Fusarium fructigenum*, *Phytophthora erythroseptica* and *Rihizoctonia solani* (crucifer).

The Table 4 shows that results for swede follow the same general trend as for potato, viz., an increase in susceptibility which increases with duration of pre-storage, and which is greater the higher the temperature. It will be noted, however, that two of the fungi used, *Pythium de baryanum* and *Rhizoctonia solani* (potato strain), failed to respond to the heat pre-treatment. A statistically significant increase of susceptibility

was obtained by the 3rd day for *Botrytis cinerea* (lettuce strain), *Phytophthora erythroseptica* and *Rhizoctonia solani* (crucifer strain) and by the 5th day for *Fusarium fructigenum*.

Table 4. *Effect of pre-storage temperature on susceptibility of* swede.—*Analysis of variance*

Due to	Degrees of Freedom	Variance Ratio (F)	Probablity
Period of Storage	3	23.40	P<0.01
Fungus	3	528.37	,,
Pre-storage Temp.	2	55.96	,,
Period × Pre-storage Temp.	6	5.68	,,
Period × Fungus	9	0.16	Not sig.
Pre-storage Temp. × Fungus	6	0.32	,,
Error	18	—	

It was noted that the order of pathogenicity on swede of the four organisms is *B. cinerea*, *P. erythroseptica*, *R. Solani* (crucifer) and *F. fructigenum* in diminishing series. The two last produce a more or less dry type of rot in contrast to the wet rot produced by the first two.

(c) *Carrot*

The results obtained for carrot in experiments conducted along the same lines as described are assembled in Table 5. With this host it was not practicable to extend the heat treatment at 35°C. beyond 5 days as longer exposure led to breakdown of the tissue and rotting by miscellaneous organisms. Of the fungi used, viz., *Botrytis cinerea*, *Rhizopus nigricans*, *Fusarium fructigenum*, *Phythium de baryanum*, *Phytophthora erythroseptica* and 2 strains of *Rhizoctonia solani*, all but the first two failed to produce attack under any conditions.

Table 5. *Effect of pre-storage at 35°C., 30°C. and 15°C. on susceptibility of* carrot

Pre-storage Temp.	Days	*B. cinerea* (Lettuce)	*R. nigricans*
35°C	1	2.195±0.20	2.320±0.14
	3	3.197±0.26	all rotted
	5	7.364±0.17	all rotted
	7	spoiled	spoiled
30°C	1	1.726±0.12	1.798±0.15
	3	2.706±0.34	3.562±0.36
	5	4.252±0.28	6.05 ±0.32
	7	5.420±0.35	14.31 ±1.12
15°C	1	1.440±0.14	1.19 ±0.11
	3	1.320±0.21	1.23 ±0.19
	5	1.375±0.11	1.29 ±0.26
	7	1.480±0.24	1.25 ±0.16

To determine whether there is any significant difference between mean of the controls (those kept at 15°C.) and treated (those kept at 30°C. and

35°C.) and also between the mean of different periods in the same temperature, Fisher's '*t*' test (1944) was applied.

In assessing the data of Table 7, it will be safe not to stress the effects produced by exposure to 35°C. as it is obvious that the temperature is dangerously high for carrot tissue. The same results are obtained as with potato and swede but in a more striking form. Thus, an increase in susceptibility to both fungi which approaches significance is obtained, even after 1 day's exposure to 30°C., and after 3 days of such exposure the rate of attack is more than doubled.

Effect of maturity of tubers on amount of attack

Inoculation experiments at different periods of growth and maturity

To observe the effect of age on the susceptibility of potato tubers to the attack of various rot-producing fungi, seven varieties of potato were used, namely, Epicure, Sharpe's Express, Arran Pilot, Great Scot, Arran Banner, Up-to-Date and King Edward. The first three are earlies, Great Scot a second early and the last three main crops. The various stages of growth and maturity at which the tubers were inoculated were as follows:

1*st. stage* i.e., when the tubers are young, undeveloped and on the average $1\frac{1}{2}''$–$2''$ in diameter; with early varieties such tubers were obtained in early July from plantings in mid April, in late varieties in the last week of July and Great Scot in mid July.

2*nd. stage* i.e., when the tubers are fairly large, but not fully developed; the tubers, in this case, were lifted 3 week after the first stage.

3*rd. stage* i.e., when the tubers are fully ripe, viz., mid to late August for earlies, mid to late September for the others.

4*th. stage* i.e., when the tubers are dormant—early to mid January.

5*th. stage* i.e., when the tubers begin sprouting—April to May.

All the above varieties were grown at the Imperial College Field Station, Slough in the summer of 1946. Thus, the same stock of material was used through the whole experiment and the possible error arising from the use of different stocks at different stages was eliminated.

The data of these experiments have been assembled in Table 6 and represent as usual the average amount of rotting produced in 10 inoculations after 6 days incubation at 20°C.

Table 8 shows that there is no significant difference in resistance between varieties tested. They behave alike so far as susceptibility to attack by the various fungi at different stages of growth and maturity is concerned. As regards the behaviour of the various fungi, the results may be summarised as follows :—

Botrytis cinerea, *Fusarium fructigenum* and *Rhizopus nigricans* which are not normally parasitic on potato tubers, actively produce rotting when the tubers are young and immature, i.e., in the 1st. and 2nd. stages and also when they are in the sprouting stage. The level of attack is comparatively low when the tubers are in the 3rd. stage and it is practically

negligible when they are dormant. Susceptibility decreases gradually from the 1st. to the 4th. stage, then suddenly jumps to a high figure when the tubers have sprouted.

The two fungi which are normal parasites of potato, namely *Pythium de baryanum* and *Phytophthora erythroseptica*, behave differently. Attack is much reduced on the younger tubers and tends to increase gradually until the dormant stage of the tuber is reached. The progressive increase is regular with *Pythium de baryanum*, less regular with *Phytophthora erythroseptica*. With both there is finally a pronounced increase in attack on the sprouted tubers.

Table 6. *Effect of maturity on susceptibility of different varieties of potato*

Variety	Stages of growth and maturity	Water Content %	*B. cinerea*	*F. fructigenum*	*R. nigricans*	*P. de baryanum*	*P. erythroseptica*
Epicure Potato	1st	84.2	0.309± 0.025	0.364± 0.01	0.318± 0.025	0.310± 0.027	0.73± 0.16
	2nd	84	0.374± 0.03	0.430± 0.03	0.359± 0.02	0.481± 0.02	1.915± 0.22
	3rd	83.6	0.287± 0.01	0.293± 0.023	0.230± 0.02	0.587± 0.02	1.533± 0.10
	4th	79.1	0.150± 0.02	0.142± 0.01	0.068± 0.02	0.720± 0.04	1.586± 0.12
	5th	77.5	0.624± 0.042	0.515± 0.03	0.36± 0.03	1.69± 0.13	4.64± 0.24
Sharpe's Express Potato	1st	80.2	0.424± 0.02	0.452± 0.02	0.331± 0.02	0.346± 0.02	0.929± 0.08
	2nd	79.6	0.501± 0.034	0.642± 0.02	0.526± 0.03	0.553± 0.02	1.92± 0.16
	3rd	79	0.278± 0.028	0.243± 0.014	0.25± 0.023	0.63± 0.04	1.622± 0.10
	4th	77	0.096± 0.01	0.108± 0.02	0.05± 0.01	0.773± 0.03	2.347± 0.25
	5th	74.2	0.787± 0.03	0.575± 0.04	0.470± 0.03	1.273± 0.08	4.829± 0.13
Arran Pilot Potato	1st	81	0.380± 0.01	0.418± 0.03	0.345± 0.05	0.40± 0.04	0.84± 0.05
	2nd	80.7	0.562± 0.025	0.731± 0.04	0.54± 0.02	0.565± 0.03	2.064± 0.26
	3rd	80.4	0.26± 0.02	0.272± 0.023	0.243± 0.025	0.607± 0.022	1.651± 0.18
	4th	78.6	0.125± 0.02	0.124± 0.013	0.04± 0.02	0.710± 0.03	2.363± 0.19
	5th	74.2	0.77± 0.03	0.536± 0.03	0.417± 0.012	1.03± 0.09	5.131± 0.28

Table 6. *contd.*

Variety	Stages of growth and maturity	Water Content %	*B. cinerea*	*F. fructi-genum*	*R. nigricans*	*P. de bary-anum*	*P. erythro-septica*
Great Scot Potato	1st	81.8	0.443± 0.03	0.48± 0.04	0.36± 0.035	0.427± 0.03	0.862± 0.05
	2nd	80.9	0.473± 0.03	0.487± 0.03	0.436± 0.04	0.05± 0.02	2.479± 0.087
	3rd	80.2	0.259± 0.026	0.224± 0.032	0.241± 0.04	0.60± 0.14	1.395± 0.13
	4th	77.2	0.102± 0.011	0.08± 0.01	0.062± 0.01	0.819± 0.093	1.883± 0.18
	5th	74	0.86± 0.04	0.60± 0.03	0.412± 0.024	1.450± 0.07	4.868± 0.35
Arran Banner Potato	1st	83.6	0.447± 0.03	0.484± 0.03	0.384± 0.02	0.388± 0.02	1.141± 0.16
	2nd	82.6	0.505± 0.03	0.538± 0.023	0.416± 0.034	0.576± 0.033	1.882± 0.16
	3rd	82	0.254± 0.024	0.286± 0.026	0.241± 0.018	0.603± 0.03	1.427± 0.12
	4th	77.1	0.121± 0.01	0.101± 0.015	0.06± 0.01	0.711± 0.11	2.016± 0.14
	5th	74.6	0.73± 0.05	0.530± 0.025	0.427± 0.02	1.251± 0.09	4.362± 0.25
Up-to-date Potato	1st	84.7	0.382± 0.016	0.401± 0.03	0.345± 0.024	0.408± 0.03	1.053± 0.15
	2nd	84	0.416± 0.032	0.495± 0.04	0.377± 0.02	0.601± 0.035	1.877± 0.15
	3rd	83	0.251± 0.024	0.255± 0.023	0.271± 0.016	0.623± 0.035	1.541± 0.17
	4th	79	0.125± 0.02	0.100± 0.013	0.07± 0.02	0.645± 0.036	1.893± 0.25
	5th	76.4	0.584± 0.03	0.513± 0.026	0.356± 0.028	1.166± 0.08	4.338± 0.24
King Edward Potato	1st	81	0.407± 0.025	0.44± 0.027	0.376± 0.014	0.409± 0.014	1.004± 0.074
	2nd	80.8	0.414± 0.209	0.593± 0.034	0.406± 0.025	0.58± 0.027	1.782± 0.17
	3rd	80	0.245± 0.023	0.278± 0.024	0.272± 0.02	0.585± 0.028	1.579± 0.05
	4th	77	0.044± 0.003	0.083± 0.015	0.026± 0.001	0.605± 0.013	1.909± 0.13
	5th	75.1	0.765± 0.042	0.591± 0.012	0.364± 0.02	2.37± 0.14	4.247± 0.15

Table 7. *Analysis of variance* of Table 6

Due to	Degrees of Freedom	Variance Ratio (F)	Probability
Variety	6	0.83	Not sig.
Fungus	4	466.8	P<0.01
Stages of Development	4	195.03	,, ,,
Variety × Fungus	24	0.34	Not sig.
Variety × Stages of Development	24	1.25	,, ,,
Fungus × Stages of Development	16	39.45	P<0.01
Error	96		

The water content of tubers gradually decreases as they pass from the 1st. to the 5th. stage. The steady fall in attack by three "non-parasites" as the tubers develop to the dormant stage may be due to the effect on these fungi on water content though this is probably not the only factor. The sudden rise as the tubers pass into the sprouting stage indicates the effect of a "maturity" factor which over rides the effect of a diminishing water content. The general behaviour of *Pythium de baryanum* accords with the effect of water content on attack by that fungus (Chona, 1932); in this case "maturity" and water content factors work together. The trend for *Phytophthora* attack is on the whole parallel to that of *Pythium*, but there are several ups and downs which would require further investigation.

Effect of dormant and post-dormant stages on susceptibility

It was shown earlier in this section that there is a considerable increase in susceptibility to rotting when the tubers pass from the dormant to the sprouting stage. It was interesting to see whether any such change takes place when dormancy is broken quite early by artificial means.

In January, 1947, dormant tubers were treated by the following methods in order to stimulate sprouting :—

(1) *Peeling method of Appleman* (1918).—The skin of the tubers was peeled off over almost the whole surface except for the region of the eyes. They were then stored in more or less dry saw dust in tin boxes at 20°C. (2) *Ethylene Chlorohydrin method of Denny* (1926).—Whole tubers were exposed to the vapour of ethylene chlorohydrin for 24 hours in air-tight glass jars (0.4 cc. of 100% solution per litre volume). After the treatment they were kept in moist saw dust in tin boxes at 20°C. (3) *Keeping in wet sawdust.*—Tubers slightly peeled at 2 or 3 places were stored in wet sawdust at 20°C. A set of controls was also kept in tin boxes at 20°C. Two different varieties were used in this connection, namely, King Edward and Majestic.

The treated tubers began to sprout vigorously within a fortnight, the sprouts reaching $\frac{1}{2}''$–$1\frac{1}{4}''$ in length. The peeling method gave the best results as regards both number and length of the sprouts. Treatment with ethylene chlorohydrin had the effect of producing a large number of short sprouts per tuber. The last method gave a few (1–2) sprouts per tuber. The controls remained unsprouted. Sprouts were not removed at the

time of inoculation The water contents of tubers after the various treatments were determined and are included in the Tables. The general conclusions to be drawn from Table 8 are as follows :

When the tubers are brought to the sprouting stage, they become more susceptible to attack than when they are dormant. There is no difference between the two varieties in this respect.

Tubers treated in the three different ways for breaking dormancy do not differ significantly from each other in susceptibility.

Table 8. *Effect of dormant and post-dormant stages on susceptibility of two varieties of potato.—Analysis of variance*

Due to	Degrees of Freedom	Variance Ratio (F)	Probability
Variety	1	1.45	Not Sig.
Treatment	3	40.03	P<0.01
Fungus	4	137.8	,, ,,
Variety × Treatment	3	0.70	Not Sig.
Variety × Fungus	4	0.001	,, ,,
Fungus × Treatment	12	3.28	P=0.05
Error	12	——	——

Physiological effects arising in certain conditions of host tissue

It is evident from the experiments described before, that under a variety of conditions there is a change in the susceptibility of the host tissue to the attack of a number of fungi, and it may be readily assumed that such change is due to some altered physiological condition of the cells leading to a higher degree of attack by various organisms. The present section records a series of experiments and observations which were designed to determine more precisely what these physiological conditions are.

Effect of temperature

Attention has been directed to the undermentioned features of the host cells, viz., (1) vitality, (2) water absorbing capacity, (3) changes in pH, (4) changes in cell-wall, and (5) changes in the diffusion of nutrients from the cells and the effect of these nutrients on germination, growth and enzyme secretion of certain fungi. These features were studied because they seemed likely to be responsible for the increase in susceptibility to rotting which results from pre-treatment of the tissue at some what high temperatures. The materials used in this connection were the potato varieties King Edward and Majestic, and swede.

(a) Changes in vitality of cells

The method of study was that of plasmolysis by 10% KNO_3, but the details were different for the two tissues, potato and swede, which were

used. The cells of potato are so full of starch inclusions that direct observation of plasmolysis under the microsope could not be carried out. Swede tissue is, however, amenable to such examination.

Shallow cylinders of potato when placed in the hypertonic solution became flaccid and pliable. On being now placed in water they became turgid and stiff, thus indicating substantial recovery from plasmolysis. By applying this test to cylinders cut from potatoes which had been stored at 35°C. for 15 days (the severest treatment adopted) it was found that the cylinders which were limp and desiccated after the treatment, became firm when placed in water. Thus, though the individual cells were not under observation, the tissue as a whole reacted in the normal living manner.

The same general conclusion can be drawn from the fact that potato tubers, even after 15 days at 35°C., later behave in a completely normal manner, e.g., they sprout as usual and on being cut across they show no abnormal features. In other words, as far as can be seen, the heat treatment adopted does not damage cells of potato tissue.

With swede, which is suitable for microscopic examination, the procedure was as follows. Two thin free-hand sections were taken from each of the three different regions of swede, namely, apex, base and centre. After being kept in 10% KNO_3 solution for some time, they were taken out and examined under the microscope. In each section, at three different places chosen at random, the number of plasmolysed cells in the field of the microscope was counted. In each particular set of observations, sections from five different swedes were taken. Examinations were made of cells of swede tissue stored at 35°C. for 1, 3, 5 and 7 days together with those of the controls stored at 15°C. for similar times. The data are set out in Table 9. The Table shows that no change in the proportion of non-plasmolysable cells had resulted from the heat traeatment until after about 5 days. At this stage, there is a slight increase which is non-significant but after a further exposure of two days the change is considerable and significant. It may be mentioned that cells from the centre of the roots are the most sensitive in this respect. Swede tissue is thus more liable to damage by heat treatment than potato tissue, as was pointed out before in connexion with studies on susceptibility to fungal invasion.

Table 9. *Effect of pre-storage on vitality of swede cells stored at* 35°C. *and* 15°C.

Pre-storage Temperature	Days				
	1	2	3	5	7
35°C.	3.15*	3.13	3.88	4.13	16.07
15°C.	3.08	3.47	3.42	3.12	3.35

* percentage of non-plasmolysed cell.

(b) CHANGES IN WATER-ABSORBING CAPACITY OF CELLS

From a cylinder of potato (1.8 cm. in diameter) ten 0.5 cm. pieces were cut, rapidly washed, dried and weighed. These were placed in distilled water for 24 hours after which they were again dried and weighed. The differences in weight expressed in terms of percentage of the first weight has been taken as the water-absorbing capacity of the cells. The results of such an experiment with three varieties of potato are given in Table 10, the figures in the Table being averages of six sets of determinations.

Table 10. *Effect of pre-storage temperature on water-absorbing capacity*

Variety	Storage at 15°C. for		Storage at 35°C. for	
	3 days	7 days	3 days	7 days
King Edward	14.10±1.35	14.4 ±1.48	15.2 ±1.56	23.2 ±1.2
Majestic	14.7 ±0.58	14.7 ±0.5	16.1 ±0.64	21.1 ±.168

A significant increase in water-absorbing capacity is thus produced after 7 days' exposure to 35°C., all the varieties behaving similarly. This effect is no doubt due largely, if not entirely, to loss of water during the heat treatment and not to any material change in the contents of the cells of the treated tissue.

(c) *CHANGES IN pH OF CELL-SAP

The H-ion concentration of the cell-sap of swede and potato stored at 15°C. and 35°C. for varying periods was determined colorimetrically. With both tissues, no change in pH of the cell sap due to storage at higher temperature was observed. The average pH of potato was 5.4–5.6 and of swede 4.2–4.4. Thus any possible role of pH in the changes in susceptibility can be ruled out.

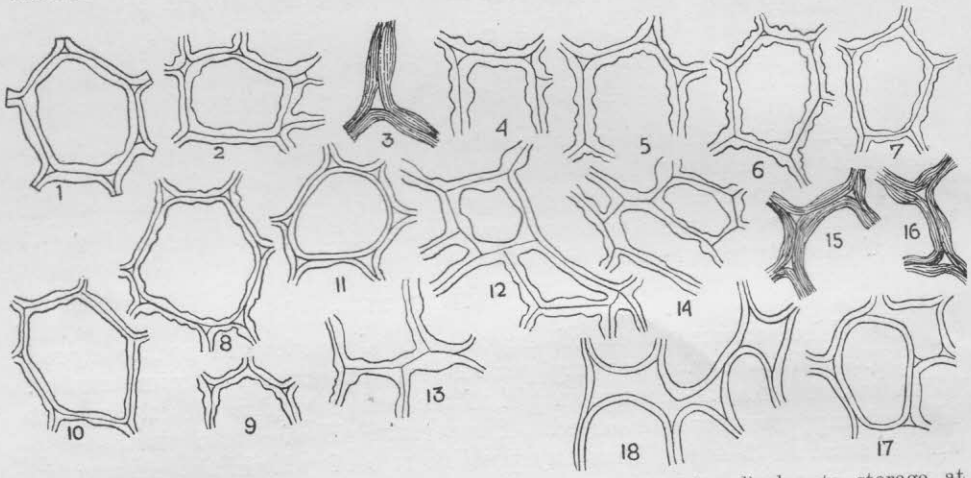
(d) CHANGES IN THE CELL-WALL

Changes in the cell-wall were studied in relation to staining reactions and to susceptibility to enzyme action.

(i) *Changes in the staining reaction of cell-walls.* Free-hand sections were cut from fresh material and stained with aqueous solution of Ruthenium Red. Pectic substances appear red after staining.

Potato. Observations were made on tissues of the varieties King Edward and Majestic which had been stored at 15°C. and 35°C. for 1–15 days. Tissue which was not exposed to the higher temperature shows, after staining, a distinct red line in the position of the middle lamella. Throughout the wall proper there is an alternation of red-stained and unstained bands. This is shown in black in fig. 3. of Text-fig.2. The walls at this stage are comparatively thick (Text-figs. 2, figs. 1–3). With tubers which are stored at 35°C., no recognisable change occurs until after about 7–10 days. Thereafter the walls become thinner in an irregular manner so that they take on a some what wavy appearance (Text-fig. 2., figs. 4-8). After 15 days storage 35°C., the walls are uniformly thin (Text fig. 2, figs. 9-11) but the distribution of red-staining material is not different from what it was at the beginning.

Swede. The cell-walls of unheated tissue stains generally and deeply with Ruthenium Red so that there is little constrast between the middle lamella and the rest of the wall. The general appearance of such a cell is shown in Text-fig. 2, figs. 12-14. When the material is stored at 35°C. changes are recognisable much earlier than with potato. Thus after 3 days, the wall ceases to stain uniformly but shows a streaked appearance (Text-fig. 2, figs. 15-16). After 7 days' treatment at 35°C., the walls are distinctly thinner and stain uniformly, but comparatively lightly with Ruthenium Red. There is also a considerable tendency of cells to break away from each other, so that the inter-cellular system is increased (Text-fig. 2, figs. 17-18). These effects are no doubt symptomatic of the breakdown which occurs after such treatment.



Text-fig. 2. 1-11, Changes in cell-wall of potato (King Edward) due to storage at 35°C : 1-3. normal, stored at 15°C. 4-8 stored at 35°C for 7-10 days. 9-11 stored at 35°C for 15 days 12-18. Changes in cell-wall of swede 12-14 normal stored at 15°C. 15-16, stored at 35°C for 3 days. 17-18, stored at 35°C for 7 days.

(ii) *Susceptibility to enzyme action.* This was tested in two ways : (1) by maceration test in which the time taken by a standard enzyme preparation to bring about loss of coherence in tissue discs of standard thickness was determined. The susceptibility is to be taken as inversely proportional to the time required for maceration. The loss of coherence was determined by taking the sections from time to time out of the solution and pulling gently with forceps. Three sections of standard thickness (1.8 cm. in diameter and 0.5 mm. in thickness) were usually placed in 3 cc. of the enzyme solution and the average time noted. In these tests, three sections from each of the ten pieces of tissues were used.

(2) by determining the amount of tissue rotted when a small quantity (0.4 cc.) of standard enzyme solution was placed in a cavity (prepared as in inoculations with spores,) and incubated for 24 hours at 20°C.

Experiments were carried out by both methods on tubers of King Edward and Majestic potatoes and by the first method on swede. For this work two different enzymic (pectinase) preparations were used, viz.,

the endoenzyme of *Botrytis cinerea* and the exoenzyme of *Pythium de baryanum*. The preparations were made in the manner already described.

With the endoenzyme of *B. cinerea*, it is possible to prepare a large quantity of powdered material so that the same stock can be used throughout the whole experiment. Thus, the possible error arising from differences in strength of enzyme preparations can largely be eliminated. With *Pythium de baryanum* this is not possible. Therefore, to have a maximum degree of uniformity, the enzyme was always extracted from inoculated potato cylinders after 15 days incubation at 20°C.

As usual the vegetables were stored at 15°C. and 35°C. and the period of storage was 1, 2, 3, 5, 7, 10 and 15 days for potato and 1, 2, 3, 5 and 7 days for swede. The results of the various observations are summarised in Tables 11–14.

Table 11. *Time (in minutes) for enzyme of* B. cinerea *to macerate discs of potato at* 35°C. *and* 15°C.

Variety	Storage Temp.	Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C	30± 0.42	26.7± 0.29	25.8± 0.53	24.2± 1.24	21.2± 0.42	20.8± 0.42	19.2± 0.68
	15°C	30.9± 0.55	30.2± 0.22	31.6± 1.73	31.4± 0.48	31.9± 0.52	31.4± 0.48	30.7± 0.64
Majestic	35°C.	33.0± 0.42	31.2± 0.44	25.8± 0.89	24.8± 0.43	23.1± 0.89	21.3± 0.91	17.5± 1.01
	15°C.	33.5± 0.55	33.7± 0.54	32.6± 0.82	33.0± 0.82	33.5± 0.47	32.9± 0.69	33.8± 0.51

Table 12. *Time for (in hours) enzyme of* P. de baryanum *to macerate discs of potato pre-stored at* 35°C. *and* 15°C.

Variety	Storage Temp.	Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	6	6	5	5	4.5	4.5	3.5
	15°C.	6	5.5–6	6.5	6	6	6	6
Majestic	35°C.	5.5–6	5.5–6	3	3	2.5–2.8	3	2.5
	15°C.	5.5–6	5.5–6	4.5	4.5	4	5	4

Table 13. *Time for enzyme of* B. cinerea *and* P. de baryanum *to macerate discs of swede pre-stored at* 35°C. *and* 15°C.

Storage Temp.	Enzyme of	Period of Storage (Days) 1	2	3	5	7
35°C.	*B. cinerea*	57± 1.3 min.	44± 1.9 min.	40± 2.0 min.	30± 1.4 min.	26± 0.8 min.
	P. de baryanum	8 hrs.	8 hrs.	7 hrs.	6.5 hrs.	6 hrs.
15°C.	*B. cinerea*	58± 2.1 min.	62± 1.7 min.	60± 0.7 min.	61± 1.1 min.	66± 3.1 min.
	P. de baryanum	8 hrs.	8 hrs.	8 hrs.	8 hrs.	8 hrs.

Table 14. *Average amount (grms.) of rotted tissue produced in 24 hours by a definite quantity of enzyme of* B. cinerea *and* P. de baryanum *on tubers pre-stored at* 35°C. *and* 15°C.

Variety	Storage Temp.	Enzyme of	Period of Storage (Days)						
			1	2	3	5	7	10	15
King Edward	35°C.	*B. cinerea*	0.315 ± 0.018	0.336 ± 0.012	0.45 ± 0.02	0.53 ± 0.025	0.57 ± 0.03	0.632 ± 0.015	0.77 ± 0.034
		P. de baryanum	0.189 ± 0.011	0.201 ± 0.007	0.251 ± 0.01	0.307 ± 0.01	0.341 ± 0.013	0.353 ± 0.008	0.44 ± 0.015
	15°C.	*B. cinerea*	0.296 ± 0.023	0.29 ± 0.017	0.288 ± 0.009	0.30 ± 0.02	0.298 ± 0.015	0.283 ± 0.008	0.286 ± 0.013
		P. de baryanum	0.177 ± 0.006	0.181 ± 0.013	0.179 ± 0.011	0.182 ± 0.012	0.189 ± 0.011	0.195 ± 0.01	0.193 ± 0.011
Majestic	35°C.	*B. cinerea*	0.294 ± 0.012	0.341 ± 0.016	0.528 ± 0.017	0.568 ± 0.026	0.744 ± 0.05	0.781 ± 0.048	0.820 ± 0.023
		P. de baryanum	0.180 ± 0.008	0.206 ± 0.007	0.291 ± 0.012	0.301 ± 0.032	0.351 ± 0.012	0.389 ± 0.027	0.499 ± 0.03
	15°C.	*B. cinerea*	0.298 ± 0.009	0.304 ± 0.013	0.281 ± 0.02	0.298 ± 0.028	0.309 ± 0.021	0.294 ± 0.016	0.294 ± 0.014
		P. de baryanum	0.176 ± 0.007	0.177 ± 0.012	0.181 ± 0.016	0.180 ± 0.012	0.184 ± 0.010	0.188 ± 0.011	0.178 ± 0.016

The experiments of Tables 11–14 agree in showing that storage of the tissues used at a temperature of 35°C. increase susceptibility to attack by the enzyme of *Botrytis cinerea* and *Pythium de baryanum*. To this effect must be ascribed a part at least of the enhanced susceptibility shown by these tissues after heat pre-treatment to attack by the organisms themselves. It is clear that the heat treatment has brought about a change in the nature of the pectic cell-wall constituents, rendering them more readily hydrolysable, and thus confirming the changes which were noted above by the staining technique. That pectinase enzymes play an important role in the hydrolysis of pectin during ripening and senescence has been observed by a number of workers, e.g., Thatcher (1915) and Atkins (1916) on the ripening of fruits, and Carré (1925) on the ripening of apple.

(e) CHANGES IN EXOSMOSIS OF NUTRIENS AND THEIR EFFECT ON GERMINATION, GROWTH AND ENZYME SECRETION OF CERTAIN FUNGI.

Increased attack by fungi is brought about when nutrient material is supplied to the spores or mycelium as was shown, for example, by Brown (1922) in experiments on the exosmosis of substances from plant structures (leaves, petals) and on the effects of those upon germination of spores of *Botrytis cinerea*. The obvious suggestion, therefore, was to test whether heat pre-treatment which has been shown to increase susceptibility has an effect on exosmosis from the cells so treated. Accordingly an investigation was taken up along the following lines : (i) estimation of total sugar, salts and total nitrogen in diffusates from host cells, (ii) influence of diffusates on germination of spores of *Botrytis cinerea*, and (iii) influence of diffusates on mycelial growth and enzyme (pectinase) secretion of *Botrytis cinerea* and *Pythium de baryanum*.

(i) *Estimation of total sugar, salts and total nitrogen in diffusates from host cells.* Sixteen cylinders (1.8 cm. in diameter and 1 cm. in height) were cut aseptically from tubers previously stored under different conditions. After being washed thoroughly with sterile distilled water to remove cell contents from the cut surface, they were transferred to 500 cc. conical flasks each containing 30 cc. of sterile distilled water. The flasks were kept at 20°C. for 24 hours after which the liquid was taken out and made up to the original volume of 30 cc. Then the amounts of total sugar, salts and nitrogen present in the liquid were determined by the following methods.

Sugar. 5 cc. of the liquid was used for this purpose. Total sugar was determined after inversion of any sucrose present, by Plank's method (1936) using the reagent of Harding and Down (1933). To correct for any reducing substances other than sugar which might be present, 5 cc. of the solution were kept at 37°C. for 24 hours with 5 cc. of yeast suspension. This removes all fermentable sugars and thus an estimate can be made by subtraction for reducing substances other than these. In actual fact, the only reducing substances present were sugars.

Salts. For this purpose, 15 cc. of the liquid was diluted with 15 cc. of distilled water and the conductivity of the solution determined by use of the "Conductivity Bridge Method" of the Cambridge Scientific Instru-

ment Company. The increase in conductivity is proportional to the increase in amount of salts.

Total Nitrogen. This was determined by the micro-Kjeldahl method with 2 cc. of the solution.

These determinations were made with Majestic potato tubers only. Two ranges of temperatures, namely 35°C. and 15°C. were used and the periods of storage were 1, 3, 5, 7, 10 and 15 days. The results in all cases represent an average of three-fold replications.

The data of the sugar estimations are given in Table 15.

Table 15. *Amount of total sugar (mg.) present in* 100 cc. *of diffusates from cells of potato* (var. Majestic)

Pre-storage Period (Days)	1	3	5	7	10	15
35°C.	27.03± 1.14	43.7± 3.8	59.8± 2.08	70.0± 3.3	89.0± 7.6	162.5± 1.5
15°C.	24.2± 0.5	25.3± 1.0	26.4± 1.1	24.2± 0.5	26.3± 1.1	26.0± 0.7

Even after 1 day storage at 35°C., exosmosis of sugar is increased, and after 3 days, the increase is highly significant. With longer exposure the rate of exosmosis becomes progressively faster.

Table 16 gives the data for total salts which have diffused out. The figures in the Table represent relative electrical conductivities ($\times 10^3$) of the diffusates.

Table 16. *Total amount of salts present in the solution expressed in terms of conductivity*

Pre-storage Period (Days)	1	3	5	7	10	15
35°C.	0.27± 0.004	0.34± 0.028	0.46± 0.032	0.52± 0.037	0.52± 0.03	1.02± 0.09
15°C.	0.25± 0.006	0.26± 0.011	0.24± 0.011	0.24± 0.011	0.25± 0.01	0.23± 0.011

The results of Table 16 follow the same lines as those for total sugar, viz., a steady increase with exposure to heat treatment. A statistically significant increase is reached by the 5th day of pre-storage. No nitrogenous substances were found in any of the solutions examined.

The leaching out of salts and sugar is no doubt primarily due to increased permeability of the plasma membrane resulting from pre-treatment at the higher temperature. Regarding sugar, there is the further possibility that under the influence of high temperature there may have been rapid hydrolysis of starch resulting in accumulation of sugar.

(ii) *Influence of diffusates on germination of spores of* Botrytis cinerea. In order to obtain diffusates in a more concentrated form than by the method

described before, the following procedure was adopted. The cylinders of tissue (1.8 cm.×3 cm. deep) were first washed as before, then each was fitted at the upper end with a sterile rubber ring arranged so as to make a water-holding chamber. Into the latter was put 0.5 cc. of sterile distilled water. The cylinders were kept in moist dishes at 20°C. After 24 hours, a drop was taken from each on a clean slide and a drop of spore-suspension added to it. The latter was so prepared that when mixed with the drop of diffusate there were about 30–40 spores in the field of the microscope. The spores were taken from cultures 10–14 days old to eliminate irregularities due to ageing. Distilled water from the same stock and treated similarly was used as controls.

The slides were incubated at 20°C. for 15–16 hours after which the average germ tube length was determined. This was based on measurements of 50 spores chosen at random from each preparation. With diffusates from potato and swede, the number of slides recorded was 8 and 5 respectively for each treatment. Tables 17 to 19 give the results of these experiments.

Table 17. *Effect of diffusates from* King Edward potato *on germ-tube length* (μ) *of* B. cinerea.

Pre-storage Period→ (Days)	1	2	3	5	7	10	15
35°C.	56.0± 0.9	63.9± 2.2	80.5± 5.1	123.3± 6.0	152.0± 4.5	175.5± 6.7	201.5± 5.0
15°C.	53.6± 1.8	49.0± 1.4	47.7± .12	50.1± 3.4	49.0± 2.2	50.0± 3.7	45.9± 2.0
Control in distilled water.	34.5± 3.6	32.0± 3.9	32.7± 1.7	30.5± 2.7	30.0± 1.0	30.1± 2.9	31.4± 2.8

Table 18. *Effect of diffusates from* Majestic potato *on germ-tube lengths* (μ) *of* B. cinerea

Pre-storage Period→ (Days)	1	2	3	5	7	10	15
35°C.	62.0± 2.1	79.0± 3.2	104.5± 3.9	118.3± 4.4	160.0± 6.0	190.4± 6.7	232.0± 7.7
15°C.	60.0± 4.0	58.7± 1.7	56.0± 2.0	60.0± 1.9	60.5± 3.4	64.8± 1.7	57.8± 2.5
Control in distilled water.	41.2± 2.0	39.5± 2.0	37.1± 2.1	40.7± 1.8	38.1± 1.3	36.9± 1.3	36.9± 1.8

Table 19. *Effect of diffusates from swede on germ-tube length* (μ) *of* B. cinerea

Pre-storage period→ (Days)	1	2	3	5	7
35°C.	124.2± 3.6	140.0± 6.5	212.4± 8.7	276.0± 10.5	368.0± 18.0
15°C.	103.6± 4.5	107.0± 2.5	105.1± 4.7	108.4± 6.4	102.1± 4.4
Control in distilled water.	39.3± 3.1	40.1± 5.1	45.5± 7.1	49.3± 3.9	51.9± 3.2

It is obvious from Tables 17–19 that water kept in contact with tissue previously stored at 35°C. has a greater stimulatory effect on the germination of spores than has water in contact with the controls. This effect is indicated after 2 days' pre-treatment of potatoes and with swede after 1 day's treatment. This enhancement in germination is progressive with lengthened period of storage at 35°C. Compared with distilled water, the liquid which was lying in contact with cells of potato and swede previously stored at 15°C. gives increased germination, but in this case, the period of storage has no effect.

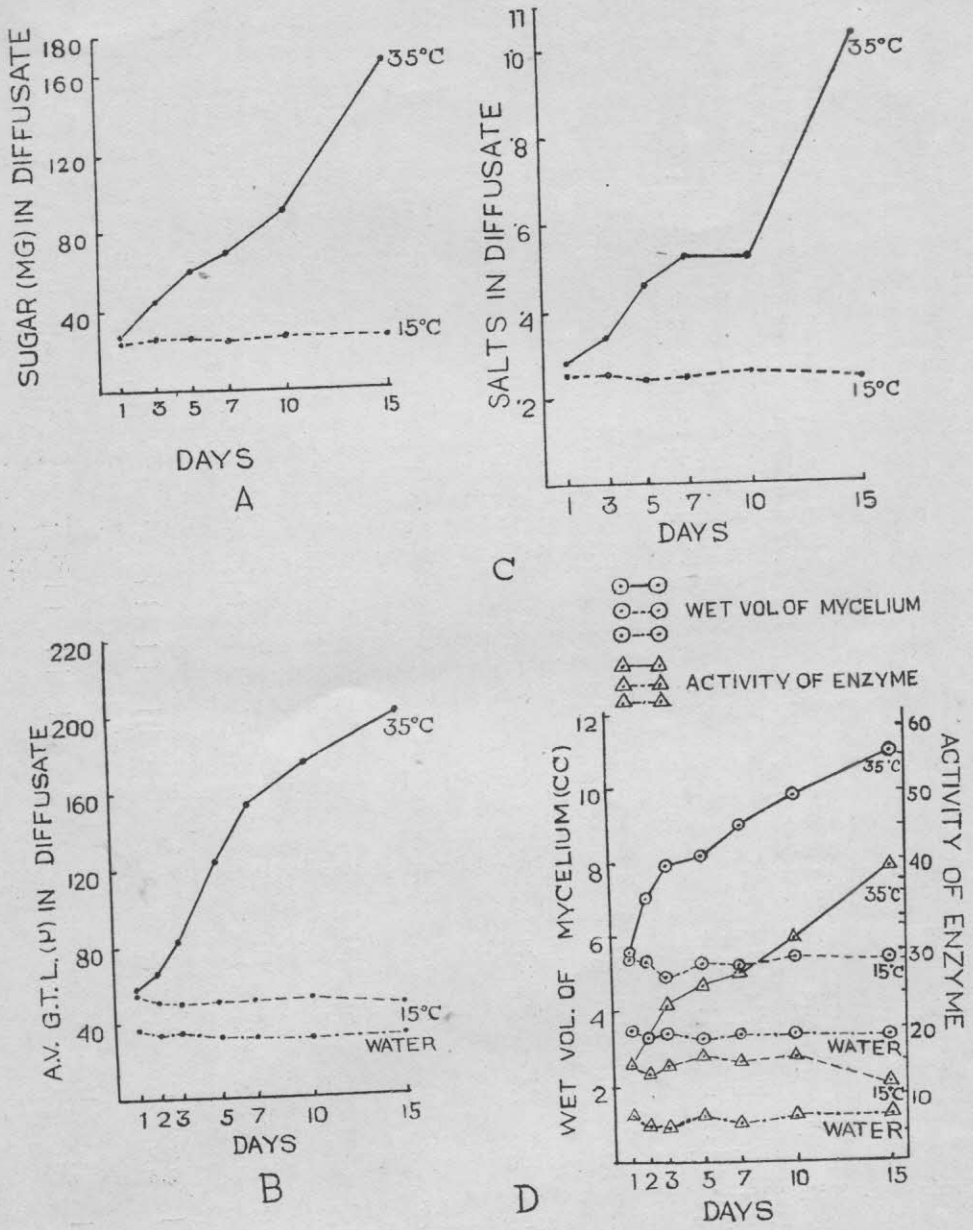
One may confidently assume that these stimulative effects on germination arise from the increased exosmosis of sugar and salts from the pre-treated tissues. The latter result has been already demonstrated by chemical and physical methods.

(iii) *Influence of diffusates on mycelial growth and enzyme (pectinase) secretion of* Botrytis cinerea *and* Pythium de baryanum. Tissue cylinders were out from control and treated potatoes and swedes and kept in 30 cc. of distilled water in 500 cc. conical flasks for 24 hours at 20°C. The liquid was then taken out and made up to the original volume.

With *Botrytis cinerea*, Brown's plate method (1915) was used for the study on growth and enzyme production. The 30 cc. of diffusate after being autoclaved was mixed with a definite quantity of spores (0.1 cc. of wet volume of spores to 10 cc. of the liquid), then pipetted on to circular glass plates (8″ in diameter) at the rate of 10 cc. per plate. After 48 hours, the liquid was taken off and the mycelium collected. As the amount of growth was small, dry weight determinations were inconvenient so that recourse was had to wet volume measurement by displacement of water. The activity of the exoenzyme was determined by the usual disc method. These experiments were run with control and treated tubers, the latter pre-stored at 35°C., and also distilled water. The last served as an additional control.

With *Pythium de baryanum*, the liquid (30 cc.) was divided equally between three 100 cc. flasks. After sterilisation the flasks were inoculated from a 4–6 day's old culture of *Pythium de baryanum* and incubated for 7 days at 20°C. The mycelial mat was then collected and its dry weight determined. The liquid was tested for the enzyme pectinase in the usual way. In this case no control with distilled water was attempted.

The results are given in Tables 20 and 21, the figures being the averages of three replicates. The activity of the enzyme preparations of *B. cinerea* has been expressed as the inverse of the time required for maceration, the activity corresponding to a maceration time of 30 minutes being taken as 100. In Table 21, which relates to *Pythium de baryanum*, the records of activity are expressed in hours.



Text-fig. 3. A. Amount of total sugar (mg.) present in 100 c.c. of diffusates from cells of Majestic potato pre-stored at 35°C and 15°C for 1–15 days. B. Average germ-tube length (μ) of *B. cinerea* in diffusates from cells of King Edward potato pre-stored at 35°C and 15°C for 1–15 days and in water. C. Amount of salts (electrical conductivity $\times 10^3$) in diffusates from cells of Majestic potato pre-stored at 35°C and 15°C for 1–15 days. D. Wet volume of mycelium (c.c.) and enzymic activity of *B. cinerea* (maceration time in hours) when grown in diffusates from cells of King Edward potato pre-stored at 35°C and 15°C for 1–15 days and in water.

Table 20. *Growth and enzymic activity of* B. cinerea *in diffusates* (*Wet volume and mycelium in c.c.*)

Potato variety	Pre-storage Temp.		Preiod of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	Mycel.	0.56	0.71	0.80	0.83	0.91	0.98	1.20
		Enzyme	13	18	21	24	25	30	40
	15°C.	Mycel.	0.55	0.54	0.50	0.54	0.52	0.55	0.56
		Enzyme	13	12	13	14	13	14	11
	Water control	Mycel.	0.35	0.34	0.35	0.33	0.34	0.34	0.35
		Enzyme	6	5	5	6	5	6	6
Majestic	35°C.	Mycel.	0.52	0.60	0.75	0.88	0.91	1.05	1.30
		Enzyme	12	15	19	25	25	32	43
	15°C.	Mycel.	0.53	0.50	0.51	0.50	0.56	0.56	0.60
		Enzyme	13	12	12	14	13	13	16
	Water control	Mycel.	0.36	0.37	0.40	0.38	0.33	0.40	0.42
		Enzyme	7	6	6	6	5	6	7
Swede	35°C.	Mycel.	1.52	2.3	3.0	3.45	3.89	—	—
		Enzyme	10	10	11	15	21	—	—
	15°C.	Mycel.	0.83	0.85	0.90	0.90	0.85	—	—
		Enzyme	10	10	11	10	10	—	—
	Water control	Mycel.	0.33	0.35	0.34	0.35	0.34	—	—
		Enzyme	5	5	5	6	5	—	—

Table 21. *Growth and enzyme activity of* P. de baryanum *in diffusates* (*Dry weight in mg.*)

Potato variety	Pre-storage Temp.		Period of Storage (Days) 1	2	3	5	7	10	15
King Edward	35°C.	Mycel.	5.3	8.4	9.0	10.3	11.0	12.7	15.6
		Enzyme	7.5	8	7	6.5	6.5	6.0	6.0
	15°C.	Mycel.	5.1	5.5	5.3	5.4	5.7	6.0	6.0
		Enzyme	7.5	8	7.5	7.5	8	8	7.5
Majestic	35°C.	Mycel.	5.3	6.0	7.4	8.3	9.6	11.3	13.7
		Enzyme	8	8.5	7	6.5	6.5	5	5
	15°C.	Mycel.	5.0	4.7	5.0	5.3	5.0	5.4	5.5
		Enzyme	8	8.5	8	8	8.5	7.5	8
Swede	35°C.	Mycel.	9	13	14.5	16.6	18	—	—
		Enzyme		20–24 hours in all cases					
	15°C.	Mycel.	7.5	8	7.6	8.2	9	—	—
		Enzyme		20–24 hours in all cases					

The general summary of Tables 20 and 21 shows that with increasing time of pre-storage at 35°C., the diffusates obtained give increased mycelial growth of *Botrytis cinerea* and *Pythium de baryanum*. Almost invariably there is a concomitant increase in the amount of exo-enzyme secreted,

the only apparent exception being in the case of *Pythium de baryanum* on swede diffusate where any variation of enzymic activity could not be established on account of the low activity shown.

Effect of age

In order to allow of comparisons being made at the same time between tubers of different ages, a batch (vars. King Edward and Majestic) was placed in cold store and thus kept in the dormant condition from early Spring 1947 until June. Meanwhile plantings were made in the field from the same stocks. In June, some of the cold-stored tubers were taken out peeled and thus stimulated to sprout. By the beginning of July, one had on hand tubers at three stages of advancement, viz., young tubers of the 1947 crop, and dormant and sprouted tubers held over from 1946. Observations were made on these with regard to cell wall and permeability changes.

(a) CHANGES IN THE CELL-WALL

Microscopic observations after staining sections with Ruthenium red did not indicate any major differences in the pectic constituents of the cell-wall in tubers of different ages. The only difference observed was a somewhat deeper staining of the walls of the young tubers.

Susceptibility to enzyme action was examined by the two methods already described, the endo-enzyme of *Botrytis cinerea* and exo-enzyme of *Pythium de baryanum* being used.

There was no difference between the tubers of different ages so far as susceptibility of cell-well to enzyme action is concerned. Thus it is indecated that changes in susceptibility of the tubers to attack at different stages of development and maturity are not due to any differences in the condition of the cell-wall.

(b) CHANGES IN THE EXOSMOSIS OF NUTRIENTS AND THEIR EFFECT ON GERMINATION, GROWTH AND ENZYME SECRETION OF CERTAIN FUNGI

The studies in this connection were carried out in the manner already described. Table 22 gives the data for exosomosis of sugar, salts and nitrogen from tubers of different ages.

The results of Table 23 indicate that much greater exosmosis of solutes takes place from young and sprouting than from dormant tubers. Further investigation would be required to determin whether this effect is due to greater permeability of the protoplasts or to a greater proportion of the cell metabolites being in a mobile condition in the form case.

The effect of the diffusates from young, dormant and sprouting tubers on spore germination is shown in Table 22 and that on mycelial growth and enzyme production in Table 24.

Table 22. *Estimation of sugar, salts* and nitrogen in diffusates (mg. per 100 c.c.) from tubers of different ages*

Variety		Young	Dromant	Sprouting
King Edward	Total Sugar	224.5± 4.0	18.3± 1.1	93.3± 4.0
	Total Salt *	3.27± 0.15	0.29± 0.02	1.08± 0.11
	Total nitrogen	Trace	Not detected	Trace
Majestic	Total sugar	185.7± 7.7	14.6± 1.1	79.4± 2.6
	Total salt *	3.05± 0.03	0.32± 0.03	0.80± 0.024
	Total nitrogen	Trace	Not detected	Trace

* conductivity $\times 10^3$

Table 23. *Effect of diffusates on germ-tube length (μ) of* Botrytis cinerea

	Young	Dormant	Sprouting	Control in water
King Edward	weft	54.0±2.3	weft	28.5±1.2
Majestic	weft	62.7±3.2	weft	30.0±1.5

The results of Tables 23 and 24, confirm those of Table 22 in showing that diffusates from young and sprouted tubers strongly surpass those from dormant tubers in their effect on fungal germination and growth. A parallel effect is seen on enzyme production, clearly in the case of *Botrytis cinerea*, less clearly in the case of *Pythium de baryanum*.

Table 24. *Effect of diffusates on mycelial growth and enzyme production*

Variety			Young	Dormant	Sprouting
King Edward	*B. cinerea*	Wet vol. of mycel. (cc.)	2.1	0.68	1.8
	P. de baryanum	Dry wt. of mycel. (mg.)	34	10	25
		Activity of exo-enyme (hrs.)	6.5	6.5	6.5
Majestic	*B. cinerea*	Wet vol. of mycel. (cc.)	2.5	0.65	2.1
		Activity of exo-enzyme	25	11	20
	P. de baryanum	Dry wt. of mycel. (mg.)	40	8	31
		Activity of exo-enzyme (hrs.)	7	6.5	3.5

COMPARISON BETWEEN *Botrytis cinerea* AND *Pythium de baryanum* IN PARASITIC BEHAVIOUR

It has been shown above that *Botrytis cinerea* does not attack dormant potato tubers to any extent, whereas *Pythium de baryanum* produces an appreciable amount of rotting. Conversely, *Pythium de baryanum* does not attack swede, or carrot, but *Botrytis cinerea* behaves as a normal parasite on these vegetables. The question therefore arises whether these differential effects are due to the presence in these various tissues of substances antagonistic to the one but not to the other of these two fungi.

Effect of plant juices on germination, growth and enzyme secretion

That antagonistic substances do exist in plant tissues has been claimed by a number of workers. Thus, Schmidt (1933) has suggested that the basis of resistance of some species of *Solanum* to *Cladosporium fulvum* is the presence of an antagonistic principle probably solanin, in the sap. Reynolds (1931) states that flax strains resistant to *Fusarium lini* contain a higher percentage of HCN producing glucoside than do suceptibile strains. Rochlin (1934) in studying the non-susceptibility of some Cruciferae to *Plasmodiophora brassicae* has observed a direct relationship between the degree of resistance exhibited by a given species and the amount of glucosides which on fermentation produce highly pungent mustard oil. Link and Walker (1933) found that sap of coloured resistant onions is more toxic to the fungus *Colletrichum circinans* responsible for the "smudge" disease of onions. Storey (1941) working with potato and radish strains of *Rhizoctonia solani* showed that resistance of Cruciferous hosts to the isolate from potato is due to the presence of some substance in the cell which the appropriate (cruciferous) strains can tolerate. Berridge (1929) states that the fresh juice of potato possesses strong agglutinating action on certain bacteria. This action is due to the presence of some substances which can be precipitated out of the potato juice by 95% alcohol at the natural pH of the sap. The precipitate solution agglutinates and plasmolyses the non-pathogenic bacteria, but pathogenic ones remain unaffected.

Raw juice of potato, swede, carrot and turnip was prepared according to the method of Menon (1934). As it was undesirable to sterilise such preparations by autoclaving or steaming, which would throw down proteins and other colloids, and as classification by means of a Seitz filter proved to be too slow or practical purposes the following method was adopted.

The crude juice was pressed from the minced tissue, collected in a sterile flask, and heated to 60–65°C. or 10 minutes. It was then held at 0°C. or 24 hours and heated again as before. This process was repeated a third time. By this means a practicable degree of sterilisation was obtained without formation of precipitates or geling of starch.

Table 25 gives the germ-tube length of *Botrytis cinerea* in juices so prepared, the figures being the average of 5 replicates.

Table 25. *Germ-tube length* (μ) *of* Botrytis cinerea *in various juices and water*

Period of Germination	Potato	Carrot	Swede	Turnip	Water
16 hrs.	200.0± 8.8	198.0± 8.7	202.0± 6.1	213.5± 6.8	58.5± 3.7
40 hrs.	weft	weft	weft	weft	—

From these results, it is clear that potato juice has no depressing effect on the germination of spores of *B. cinerea*.

The amount of mycelial growth of *B. cinerea* and *P. de baryanum* in the various juices was determined by growing each fungus in 10 cc. of the liquid in 100 cc. flasks. The experiment was run in triplicate at 20°C. for 7 days. The data obtained are given in Table 26.

Table 26. *Mycelial growth* (*dry weight in gms.*) *of* B. cinerea *and* P. de baryanum *in various juices*

	Potato	Carrot	Swede	Turnip
Initial pH	5.4	5.2	4.4	4.4
B. cinerea	0.45± 0.03	0.56± 0.07	0.60± 0.04	0.52± 0.05
P. de baryanum	0.35± 0.02	0.15± 0.03	No growth	No growth

Thus *Botrytis cinerea* can grow well on all the plant juices. On the other hand, *Pythium de baryanum* grows well on potato and to a moderate extent on carrot, but not at all on swede or turnip. The absence of growth in the last two cases may be due to the low pH, because *Pythium de baryanum* is not favoured by acidity of the medium.

The ability of the fungi to secrete enzyme and the strength of the enzyme if secreted, were studied in the following ways :—

(1) With *Botrytis cinerea*, Brown's plate technique (1915) was adopted, After 48 hours' growth, the activity of the endo- as well as the exo-enzyme was determined in the usual way.

(2) With *Pythium de baryanum*, after 7 days' growth at 20°C., the liquid was tested for exo-enzyme.

Table 27 gives the result of the experiment. The strength of the enzyme has been expressed in terms of time taken to macerate the discs of potato. A 3-fold replication was used.

The outstanding result from Table 27 is that *Botrytis* produces a much weaker exo-enzyme on potato than on carrot and that the exo-enzyme of *Pythium* behaves in a converse manner.

Table 27. *Activity of enzyme secreted by* B. cinerea *and* P. de baryanum *in various plants juices*

	Potato	Carrot	Swede	Turnip
Initial pH	5.4	5.2	4.4	4.4
B. cinerea (exo.)	12 hrs.	1.5 hrs.	48 mins.	53 mins.
B. cinerea (endo.)	1.5 hrs.	45 mins.	35 mins.	35 mins.
P. de baryanum (exo)	3.5 hrs.	18–20 hrs.	No enzyme as there was no growth.	As for Swede

Effect of plant juices on enzyme activity

The results just described may be due in part to different rates of excretion of enzyme according to the medium used, but they are also to be explained (in part at least) by the fact that different plant juices have unequal retarding effects on the macerating action of the enzyme. This is shown by the results of Table 28. An enzyme solution was set up and a series of dilutions was made: (1) with water, (2) with various juices, and the activities of these dilutions compared.

Table 28. *Effect of plant juices on the activity of enzyme of* B. cinerea *and* P. de baryanum

Enzyme	Conc.	Diluting Liquid				
		Water	Potato	Carrot	Swede	Turnip
B. cinerea (endo.)	100%	35 mins.	—	—	—	—
	75%	40 ,,	130 mins.	70 mins.	65 mins.	65 mins.
	50%	55 ,,	11 hrs.	130 mins.	120 mins.	130 mins.
	25%	80 ,,	24 hrs.	300 mins.	280 mins.	320 mins.
P. de baryanum (exo.)	100%	210 mins.	—	—	—	—
	75%	260 ,,	300 mins.	8 hrs.	8 hrs.	8 hrs.
	50%	310 ,,	450 mins.	24 hrs.	22 hrs.	22 hrs.
	25%	480 ,,	11 hrs.	24 hrs.	24 hrs.	24 hrs.

Similarly, when extractions of enzyme powder were made int he same ratio in water, potato, carrot, swede and turnip juices, the times for maceration of the resulting preparations were 35 mins., 12 hrs., 150 mins., 130 mins., and 130 mins. respectively.

The following conclusions emerge from the above data :—(i) Plant juices, in general, have a retarding effect on the activity of the enzyme as shown by Brown (1915). (ii) The endo-enzyme of *Botrytis cinerea* is particularly affected by the raw juice of potato. The activity of the enzyme extracted in raw juice of potato is also low. (iii) With *Pythium*

de baryanum, potato juice does not have a great retarding effect, but juices of carrot, swede and turnip have strong inhibitory influence. These observations confirmed the earlier work of Chona (1932) on the differential effect of potato and turnip juices on enzymes of *Botrytis cinerea* and *Pythium de baryanum* and offer a line of approach to an investigation of the differential behaviour of these fungi on these hosts.

Behaviour of the fungi on living potato tissue

Inoculation experiments were carried out on cylinders of potato var. King Edward with *Botrytis cinerea* and *Pythium de baryanum* to examine in detail the course of events leading to unsuccessful or successful attack as the case might be. The method of procedure was that explained before. With *B. cinerea*, the inoculum was either a spore-suspension or a piece of mycelium with *Pythium de baryanum* the latter only. After inoculation, the cylinders were examined at 24 hours intervals for three successive days. The observations were made mainly on freehand sections of the fresh material which were stained with cotton blue. The effect is to stain the spores and mycelium blue against the colourless background of the cells. Scharlarch R. or Sudan III was used for the test of suberin. For detailed studies, the material was fixed in Craft A and B, and embedded in paraffin. The microtome sections (12–14μ thick) were stained in Haemotoxylin with Sudan III as counter stain (Dey, 1919). The results of the observations may be summarised as follows :—

With cylinders of potato inoculated with spores of *B. cinearea*, germination occurred freely within 24 hours and the germ tubes grew quite well. There was nothing to suggest any checking of the growth of the fungus at this stage. The fungal hyphae attacked the cells and a small amount of rotting was produced. Usually only a few layers of cells were involved; but after about 3 days, the presence of suberin in the walls of cells underlying the rotted tissue could be demonstrated. Similar suberisation was seen when mycelial inocula were used. It may be that the cork layer so formed constituted a mechanical barrier which led to stoppage of invasion, but it is equally plausible that invasion had been stopped by other factors and that cork-formation was the normal response of the healthy underlying cells to a wound stimulus.

With *Pythium de baryanum*, a progressive and gradual rotting occurred. The rotting was rapid to start with and no suberised layers were found to be present.. The main conclusion to be drawn from these observations is that germination of the spores is normal and that the factor which confers resistance comes into play after germination has taken place.

Behaviour of the enzymes on living potato tissue

Fernando and Stevenson (1952) have suggested that the enzyme of *Botrytis cinerea*, when placed on potato tissue, is unable to produce much rotting because it is rapidly absorbed by the cells of the potato. A series of experiments was carried out in which attention was paid to the rapidity with which a given small quantity of enzymic solution was absorbed into the tissue, in order to see whether there was any relationship between the speed of absorption and the amount of rotting produced.

The cylinders (1.8 cm. in diameter and 3 cm. in length) were cut from the tubers of var. King Edward and a small cavity (0.5 cm. in diam. × 1 cm. deep) made on the top of each cylinder. After thorough washing, small measured quantities (0.2 cc.) of enzymic solutions were placed in the cavities and the material was then incubated in moist dishes at 20°C. The results are assembled in Table 29 which gives the particulars of the various enzymes and enzyme mixtures (equal volumes) used. The figures represent the average of ten replicates.

Table 29. *Behaviour of various enzyme preparations of* B. cinerea *and* P. de baryanum *on potato tissue*

Enzyme	Time for maceration (in mins.)	Amt. (gm) of rotting of cylinders	Time for Absorption of 0.2 cc. of Enzyme Solution (hrs.)
1st Set			
1. *Botrytis*, endo. in water	25	0.066± 0.006	18
2. *Botrytis*, endo. in water deact	—	—	16
3. *Bot.*, exo. in Turnip Extract	50	0.057± 0.007	24
4. *Bot.*, exo. in Turnip Extract deact.	—	—	24
5. *Bot.*, endo. (water) *Bot.* exo. (T.E.)	35	0.065± 0.007	20
6. *Bot.*, endo. (water) *Bot.* exo. (T.E.) dact.	—	—	20
7. *Pythium*, exo. in Synthetic Soln.	25	0.28± 0.002	20
8. *Pyth.*, exo. in Synth. Soln. deact.	—	—	16
9. *Bot.*, exo (T.E.) *Pyth.* (exo.) in S.S.	35	0.13± 0.01	24
10. *Bot.* (exo.) (T.E.) *Pyth.* (exo.) in S.S. deact.	—	—	24
11. *Bot.* (endo.) (water). *Pyth* (exo.) in S.S.	25	0.143± 0.023	16
12. As above 11—deact.	—	—	16
13. *Bot.*, endo. in 7.5% Sucrose Soln.	75	0.07± 0.003	Not absorbed
14. *Bot.*, endo. in 15% Sucrose Soln.	105	0.07± 0.007	Not absorbed
15. *Pythium* exo. from Cylinders of Potato	270	0.05± 0.005	24
16. As 15 above, deact.	—	—	24
17. *Bot.* endo. (water) *Pyth.* (exo.)	150	0.09± 0.008	20
18. As 17 above, deact.	—	—	20

Table 30. *Progress of attack on potato tissue by various enzyme preparation of* B. cinerea *and* P. de baryanum

Enzyme	Time for maceration (mins.)	Amount of rotting of cylinders after 24 hours	48 hours	72 hours	Time for absorption of the enzyme solution (hrs.)
1st Set. (0.1 cc. of enzyme used)					
1. *Botrytis* endo.—(in water)	35	0.069± 0.003	0.070± 0.003	0.075± 0.009	16
2. *Botrytis* exo.—(in Turnip Extract)	45	0.069± 0.003	0.71± 0.009	0.079± 0.009	24
3. *Pythium* exo.—(in Synthetic solution)	45	0.213± 0.01	0.288± 0.014	0.352± 0.014	16
4. *Botriytis* endo.—(water) *Bot.* exo.	40	0.072± 0.004	0.081± 0.01	0.90± 0.007	24
5. *Botrytis* endo.—(water) *Pyth.* exo.	40	0.135± 0.012	0.180± 0.006	0.225± 0.012	16
6. *Botrytis* exo.—(T.E.) *Pythium* exo.	45	0.126± 0.012	0.187± 0.012	0.272± 0.011	24
2nd Set. (0.2 cc.) of enzyme used)					
7. *Botrytis* endo.—(in water)	30	0.107± 0.007	0.114± 0.09	0.128± 0.005	19
8. *Botrytis* exo.—(in Synthetic solution)	25	0.125± 0.008	0.132± 0.013	0.139± 0.012	20
9. *Pythium* exo.—(from inoc. cylinders of potato)	210	0.087± 0.01	0.112± 0.013	0.200± 0.018	24
10. *Botrytis* endo.—(water) *Botrytis* exo.	25	0.130± 0.012	0.138± 0.011	0.148± 0.016	20
11. *Botrytis* endo.—(water) *Pythium* exo.	45	0.087± 0.01	0.162± 0.013	0.208± 0.018	24
12. *Botrytis* exo.—(Syn Soln.) *Pythium* exo.	45	0.089± 0.01	0.156± 0.014	0.222± 0.027	24

The conclusions drawn from the above Table are as follows:—(i) *Botrytis* endo-enzyme when prepared in water (No. 1,) is absorbed in about 16–18 hours and produces no more than about 0.06 gm. of rotted tissue even when in high concentration (i.e., with maceration time about 25–30 minutes). (ii) *Pythium* enzyme of the same activity in maceration test (No. 7) in absorbed in much the same time but gives much increased rotting (0.28 g.). (iii) *Botrytis* enzyme of high activity but with osmotic contents present (No. 13, 14) is not absorbed, but yet gives little rotting (0.07 gm.). (iv) With preparations of low activity in the maceration test a comparatively greater amount of rotting is caused by *Pythium* enzyme than by *Botrytis* enzyme. (v) Enzyme mixtures give intermediate data as compared with each constituent. (vi) The rate of absorption of deactivated is the same as that of active enzyme. It appears, therefore, that the source of the enzyme i.e., whether from *Botrytis* or *Pythium* combined with its intrinsic activity as measured by the maceration test determines the amount of rotting which takes place. The time required for absorption by the tissue is not obviously of any significance.

The progress of attack with various enzymic preparations, was further examined in the experiments recorded in Table 30 and the following general conclusions may be drawn :—(i) With the enzyme of *Pythium de baryanum*, the rotting of potato cylinders is progressive with increasing time. This is shown clearly in cases of both stronger (No. 3) and weaker (No. 9) preparation. (ii) With the enzyme of *Botrytis cinerea*, both exo.- and endo.- the same tendency is evident, but in no case do the increases come up to the level of significance. This is more marked when a larger quantity is used (Nos. 7 and 8) than with a small quantity (Nos. 1 and 2). (iii) The mixtures, as before, behave intermediately between the two components.

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