STUDIES IN THE LATENT PHASE OF COLLETOTRICHUM SPECIES CAUSING RIPE ROTS OF TROPICAL FRUITS

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SUMMARY

The factors underlying the development of latent infections by species of *Colletotrichum* causing ripe rots in tropical fruits were investigated with respect to the four aspects of nutrition, enzyme activity, toxins and respiration. Most of the work was carried out with *Gloeosporium musarum* on the Cavendish banana.

Failure of *G. musarum* to develop in green fruit is not due to inability to utilize starch nor is a deficiency of the simple sugars a limiting factor. The addition of nutrient solutions containing highly organized nitrogenous compounds leads to accelerated development in the inoculated green banana.

Species of *Colletotrichum* appear to have a poor capacity for secreting macerating enzymes and pectinesterase compared with *Penicillium digitatum* and *Rhizopus nigricans* when grown on similar media. This capacity is augmented by increasing the nitrogen content of the media. The addition of "Pectinol" or the solutions from liquid cultures of *Colletotrichum* and other fungi effects a varying acceleration of *G. musarum* development. By heating these solutions to destroy enzyme activity it was shown that the action could be a nutritive one rather than enzymatic.

No anti-fungal substance of the phytoalexin type could be demonstrated in the banana nor was a toxic reaction exhibited by cold extracts of the green peel. Hot aqueous extracts of the outer peel, using commencing temperatures of 80°–95° C, were highly toxic. Neither cool nor hot extracts of ripe peel showed toxic properties. It was possible to obtain toxic extracts from other fruit subject to latent infection and some circumstantial evidence was obtained suggesting that a toxic substance may be one factor in determining the state of latency. Confirmation must await the results of more detailed investigations.

The respiratory changes taking place in ripening fruit at the time of the climacteric were compared with those induced by parasitic attack and the hypothesis put forward that the former provides a metabolic environment assisting fungal development in the host. With respect to this hypothesis, the effect of a number of reagents on the development of *G. musarum* in bananas was investigated. Those substances known to produce a marked effect on metabolic activity, such as DNP and 2,4-D, were usually associated with accelerated fungal development. The reverse holds for white oil, benzimidazole and kinetin.

The influence of wounds in stimulating *Colletotrichum* development was attributed to metabolic changes simulating ripening which take place in the adjacent tissue as a result of wound stimulus.

The paper concludes with a suggested interpretation of the development of *G. musarum* in relation to the environment provided by the green and the ripe banana.

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I. INTRODUCTION

The ripe rots constitute a common and serious loss in tropical fruits the world over. Because rotting may still occur in fruit which have been surface sterilized, it was early assumed that the pathogens concerned were able to penetrate the skin of green fruit and remain there in a quiescent or latent condition only to regain activity as ripening progressed. This early work has been reviewed by Baker, Crowdy, and McKee (1940).

The organisms most consistently involved in the ripe fruit rots belong to the genus *Colletotrichum*. (When common usage dictates, the synonym *Gloeosporium* is still used in this article). In a paper complementary to the present one, Simmonds (1941) showed by field inoculations that the anthracnose fungus

(*Gloeosporium musarum* Cke. & Mass.) could remain latent within the skin of the green banana fruit for almost five months and then produce the typical rot as the fruit ripened. It was also demonstrated histologically in the banana, papaw and mango that the form taken by the fungus during the latent period is a small knot of mycelium, the subcuticular hypha, arising from the base of the appressorium and lying beneath the cuticle or within the cellulose layers of the outer cell wall. This was confirmed for the banana by Chakravarty (1957), while Fulton (1948) and Illman, Ludwig, and Farmer (1959) have described a similar relationship between *C. phomoides* and *C. atramentarium* and their tomato host. According to Adam *et al.* (1949), the infection thread of *C. gloeosporioides* in the orange (a non-climacteric fruit) does not rest below the cuticle but penetrates intercellularly the upper two or three layers of cells.

As the physiological changes associated with ripening take place the subcuticular hypha resumes activity and commences to invade the tissue as an intercellular hyphal thread. Later, cell penetration may occur and about the time the first visual brown speck appears on the surface an extensive inter- and intracellular mycelium has developed (Simmonds 1941; Chakravarty 1957; Meredith 1960). The effect of a number of factors connected with the growing, harvesting and marketing of Cavendish bananas on the incidence of anthracnose and finger stalk rot caused by *G. musarum* was investigated by Simmonds and Mitchell (1940).

Perhaps the most interesting feature in connection with the latent infection is the question of the factor responsible for restraining the activity of the fungus in the green fruit but which becomes inoperative as the fruit ripens. Simmonds (1941) discussed this question in the light of existing knowledge and other workers have made tentative suggestions from time to time, but no fully substantiated explanation has been forthcoming. The work described in this paper is an attempt to provide further information on the subject of latency with the object of getting nearer to a satisfactory theory.

Broadly speaking, there are four main propositions which can be offered to explain the latent state:

(1) The nutritional requirements of the parasite are not met by the composition of the green fruit.

(2) The enzyme potential necessary for invading the green fruit is greater than for the ripe and is temporarily beyond the capability of the fungus.

(3) There is a toxin present in the green fruit which disappears or is inactivated in the ripe.

(4) The energy requirements of the fungus can only be met when the metabolism of the host has passed from the green to the ripening phase.

There could well be an interaction of two or more of these factors but for simplicity they will in the first instance be treated separately and correlation will then be attempted in a general discussion. The work described has been carried out at disjointed intervals over a number of years. An attempt was made to obtain a broad view of the subject rather than to make a detailed examination of one facet without relation to the whole. In consequence, some of the work would benefit from further elaboration. Some of the implications are a subject for the specialist physiologist or biochemist. Citations are made to illustrate or support a point under discussion and are not intended to be all-inclusive.

II. THE CIRCLE INOCULATION METHOD

The basic data used in this investigation have been obtained from the effect various substances have on the development of *G. musarum* in banana fruit. The banana was selected because of its ease in manipulation. Also, fruit can be obtained all the year round and when ripening they show a clearly defined climacteric. Freely fruiting cultures of *G. musarum* are readily available. The Cavendish variety or one of its taller mutants has been used almost exclusively.

The circle inoculation technique described in a previous paper (Simmonds 1941) has been used to a large extent. From a single bunch in the hard green state the fingers are separated and cleansed by wiping with 50 per cent. alcohol. On the most convenient side of the fruit, three or four circles approximately 1 cm in diameter are drawn with a camel-hair brush and Indian ink. For checks, one or two squares of similar size are also included. These are left uninoculated and serve to indicate any phytotoxic properties the solutions may possess. The circles are inoculated by spreading over each a drop of a faintly cloudy spore suspension containing approximately 3,000 spores per drop The squares at this time receive sterile water only.

The fruit are incubated in moist chambers for 24 hr or somewhat longer, when the subcuticular hyphae are assumed to have been formed. Germination and appressoria formation are checked in a slide preparation or by direct examination of thin slices taken from an inoculated circle. The circles and squares are next dried and then prepared to receive the test solutions by making a series of light pricks with a battery of pins, using 40–50 pricks to each. These pricks allow absorption of the test solution but are not sufficiently extensive to serve as wounds and thus accelerate development on their own.

Drops of the test solution are applied to both circles and squares and the fruit are returned to the moist chambers. Most solutions are absorbed readily and the drops are topped up once or twice daily for two or three days. With some compounds, when a toxic action on the spores is anticipated the test solution is applied before inoculation.

At the conclusion of the experiment, usually when the fruit have attained the fully ripe stage, the diameter of the lesion developed is measured. As the actual size of the lesion produced by any one treatment varies according to a number of factors, such as the ripening peculiarities of the bunch used and the temperature of incubation, the effect of the treatment is assessed in relation to the lesion produced on inoculated circles by sterile water checks included in each experiment. A further check on the conditions of the experiment is obtained by including a 1 per cent. or 2 per cent. solution of "Pectinol 100 D." This proprietary enzyme preparation produced a marked and uniform acceleration of lesion development and provided a standard of comparison in the upper ranges.

The following symbols are used to designate the extent of lesion development:

– Less than the sterile water check.

o Approximately the same as the water check.

\+ Greater than the check but not markedly so.

++ Markedly greater than the check.

The application of these categories is illustrated in Figure 1. Usually in any one series two fruits were used for each treatment and in most cases duplicates showed uniform development.

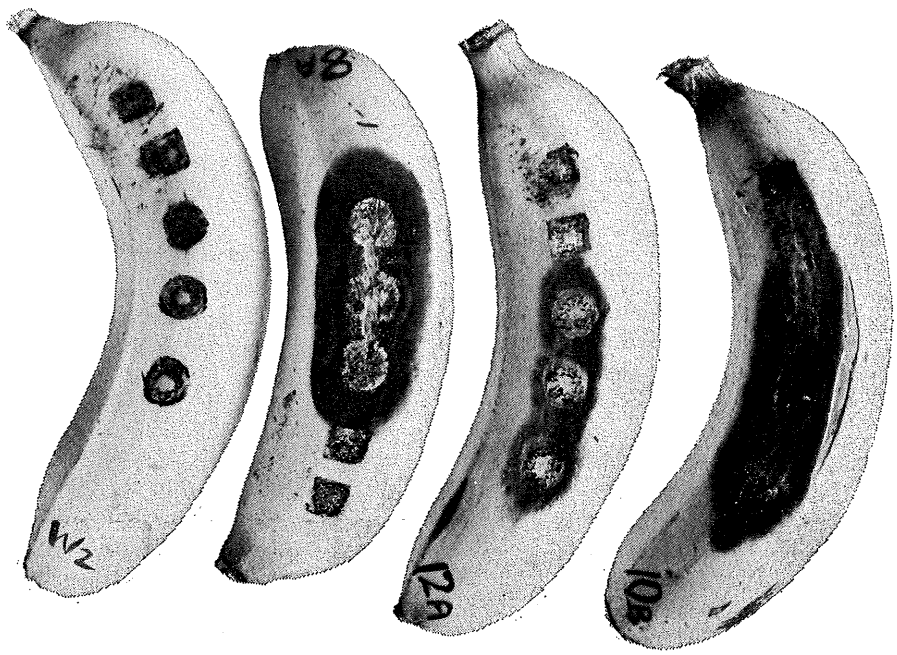

Fig. 1.—Illustrating the method of rating in the circle inoculation tests. W2, sterile water "O"; 8A, sodium benzoate 0·25% "++"; 12A, Pectinol 2% autoclaved 15 min "+"; 10B, Pectinol 2% "++".

It is well known that wounding green fruit may accelerate anthracnose development by several days and therefore an applied chemical having a phytotoxic action may for this reason alone accelerate lesion development. Similarly, a toxic action on the fungus itself will retard or even eliminate development. An endeavour has been made to use concentrations which would avoid such interference, but when applicable notes on phytotoxicity and spore germination tests have been included in the tables. Solutions designated "highly fungitoxic" completely inhibited spore germinaton. Others causing reduced germination and germ-tube growth were designated moderately or slightly fungitoxic as the case might be. Inhibition of germination does not necessarily mean death, as subsequent transfer to a nutrient solution will often show.

The existence of a macerating effect was investigated in most cases and for this purpose the solutions were subjected to a potato slice test based on the procedure described by Brown (1915). Rectangular blocks of potato tuber tissue approximately 2·0 x 1·0 cm were cut on a hand microtome into slices 0·5 mm thick. Two or three of these were immersed in 10–15 ml of solution and tested manually from time to time for coherence. The time taken to reach a state of disintegration or complete lack of cohesion was noted and this time has been included where appropriate in the tables. A Pectinol solution was included in these tests as a check.

III. THE NUTRITION HYPOTHESIS

von Loesecke (1949) attributes to Kervegent the statement that the diastatic enzymes secreted by *Gloeosporium* have an insignificant action on starch granules so that the fungus attacks the fruit on the verge of maturity when most sugar is present. This is a simple and easily accepted explanation and one which has been put forward from time to time to explain latent infection. It is applicable to all those fruits in which starch is the major carbohydrate reserve in the green stage. The papaw is a notable exception since it contains no starch and there is apparently an ample supply of sugar in both the green and the ripe state—72 and 79 per cent. respectively of the dry weight (Jones and Kubota 1940).

Since this argument rests on the extent to which starch can be utilized by the fungus, this point was investigated. A basal media (asparagine 0·2 per cent.; $MgSO_4$ 0·075 per cent.; K_2HPO_4 0·125 per cent.) minus carbohydrate was prepared based on Brown's synthetic media. Potato starch, dextrose, pectin and cellulose at 3 per cent. concentration were autoclaved separately. A spore suspension of the test fungus was made up in the basal media and 10 ml of this added to 20 ml of the carbohydrate in petri dishes. These were incubated at 27°C for eight days, when the mats were removed, dried on weighed filter paper and the weight of each mat determined. This experiment was replicated four

times, using four plates for each medium in each replication. The results are given in Table 1. The figures for cellulose are omitted owing to the difficulty of separating the mat from the media in some instances. Cellulose was not a good substrate for growth.

TABLE 1

AVERAGE COLONY WEIGHT (IN MG) OF THREE FUNGI USING DEXTROSE, STARCH AND PECTIN AS THE CARBOHYDRATE SOURCES

Organism	Dextrose (pH 7·8)	Starch (pH 8·1)	Pectin (pH 3·5)
Gloeosporium musarum	207	244	143
Gloeosporium sp. (strawberry)* ..	255	201	197
Botrytis cinerea	257	178	139

s.e. 9·8. Necessary differences for significance, 28 (5%), 38 (1%)

* See page 389

Under the conditions of this experiment *G. musarum* grew better on starch than on dextrose. For another trial, using a basal medium based on Czapek's solution with half the amount of nitrogen and adding 2 per cent. of the appropriate carbohydrate, *G. musarum* grew slightly better on starch than on sugar, while the other two preferred glucose as before.

Other workers have investigated the carbohydrate nutrition of the Gloeosporiums. Grewal (1957), working with *G. musarum* and a species of *Gloeosporium* and of *Colletotrichum* from papaw, found that starch supported less growth than either glucose or sucrose. Thind and Rawla (1958) obtained similar results with three other species in these two genera. On the other hand, the three species of *Gloeosporium* used by Tandon and Agarwala (1956) produced somewhat better growth on soluble starch than on either glucose or sucrose except in one instance when glucose proved a slightly better substrate.

The important fact arising out of these experiments is that irrespective of preference all species were able to make good growth on starch as the sole source of carbohydrate. Furthermore, if a spore suspension of *G. musarum* is applied to the starchy pulp of a green banana on an area from which the skin has been removed, vigorous and abundant growth takes place (Table 19, Figure 6).

To explain latent infection in the banana (and probably other fruits) in terms of the inability of the parasite to utilize starch is therefore an unjustifiable assumption. This does not preclude the possibility of other more complex nutritional factors operating, and information obtained from the fruit inoculation tests will now be discussed.

Referring to Table 2, it will be seen that there was no suggestion that either dextrose or sucrose was in critical supply. Addition of these sugars in various concentrations had no effect on development. This is contrary to the results obtained by Sitterly and Shay (1960), who were able to advance by 20 days tissue invasion of the apple by *Glomerella cingulata* by infusing sucrose and fructose through the spur leaf petioles.

TABLE 2

EFFECT OF VARIOUS SOLUTIONS APPLIED TO INOCULATED GREEN BANANAS ON ANTHRACNOS DEVELOPMENT

(a) Nutrients

Material and Concentration	Rating	No. of Trials in Which Used	Comments
Dextrose 5%; 3%	0	1; 2	
Dextrose 1·5%; 1%	0	1; 1	
Sucrose 5%; 1%	0	1; 1	
Asparagine 1·0%; 0·75%	0	1; 1	
l-leucine 1·0%	0 (1); + (2)	3	
l-leucine 0·1%	0	1	
dl-serine 1·0%	0 (1); + (2)	3	
dl-serine 0·1%	0	1	
l-valine 1·0%	0/+	2	
l-valine 0·1%	0/+	1	
Pectin 1·5%	+	1	
Casein (soluble) 1%	+	4	
Casein hydrolysate 1%; 0·5% ..	+	1; 1	
Casein hydrolysate 0·1%	0	1	
Potato infusion 15%	0	2	Slightly fungitoxic
Potato infusion 15% (heated) ..	+ (3); ++ (1)	4	Steamed 15 min (2); autoclaved 15 lb (2)
Potato infusion 10% (heated) ..	0	2	Steamed 15 min (1); autoclaved (1)
Medium A* (low organic nitrogen) ..	+ (4); +/++ (2)	6	
Medium B† (nitrate nitrogen) ..	+/++	2	
Medium C‡ (high organic nitrogen) ..	++ (3); ++/+ (2)	5	
Pepsin 2%	++	5	Nil macerating ability
Pepsin 2% (heated)	++	5	Steamed 15 and 60 min (2); autoclaved (3)

* Citrus pectin 0·5%; dextrose 0·5%; starch 2·0%; asparagine 0·1%; peptone 0·1%; soluble casein 0·1%; magnesium sulphate 0·05%; potassium phosphate tribasic 0·2%

† As A, except nitrogen in form of sodium nitrate 3%

‡ As A, except asparagine, peptone and casein each at 3%

The nitrogenous compounds were more active than the sugars and the accelerating action increased with the complexity of the solution used. Pepsin and the high-nitrogen media consistently produced marked acceleration. As will be seen from the next section, if the fungus has the opportunity of adding to this medium the products of its own growth an even greater effect is produced.

Pepsin was used in the first place because of the suggestion of Ginzburg (1958) that there might be a protein constituent of the middle lamella which has to be removed before the pectates are accessible to enzyme action. However, the solution showed the same activity after being autoclaved at 15 lb for 20 min or steamed for up to 60 min, and it is evidently in a nutritive capacity that it is functioning here.

The dependence of fungi on specific nitrogenous compounds for their growth and parasitic activity is well known and has been the subject of considerable investigation. Tandon and Grewal (1957) list the relative growth rates of *G. musarum, G. papayae* and *C. papayae* on a number of nitrogenous compounds and show that large differences may be expected. Vasudeva (1961) records that the addition of a nitrogen source, organic or inorganic and including the amino acids argenine, valine, leucine and asparagine, at the site of inoculation tends to increase the spread of *Colletotrichum falcatum* in sugar cane. Ashour (1954) and others have shown that the concentration of the nitrogen fraction of a culture media may have a marked influence on enzyme secretion and this is not necessarily related to vegetative growth.

Two other references are specially pertinent. Vasudeva (1930) induced *Botrytis allii* to parasitize immature apple tissue by adding to the inoculum a nitrogenous compound. The amount of nitrogen required diminished as the fruit ripened and when they were fully ripe invasion occurred without any addition. *B. allii* growing on apple juice alone produced no observable macerating enzyme but when asparagine or potassium nitrate was added an appreciable quantity formed.

Kline *et al.* (1957) have shown that certain biochemical mutants of *Venturia inaequalis* possessing specific nutritional requirements were able to penetrate the cuticle of the apple leaf and establish themselves in a typical subcuticular position in the absence of this particular substance but were unable to develop further until it was supplied as a supplement to the leaf surface. The supplements consisted of amino acids and other nitrogen compounds. They concluded from their experiments that in most cases the required substance was already present in the host but apparently not in sufficient concentration at the site of infection to meet the needs of the parasite. Similar results were obtained by Dutta, Hall, and Heyne (1960) working with *Colletotrichum lagenarium* on cucurbits. On the other hand, no evidence was obtained by Williams (1960) that host specificity in wild-type lines of *V. inaequalis* is due to simple differences in amino acids between susceptible and resistant varieties of apple.

Lewis (1953), in supporting his balance hypothesis of parasitism, pointed out that the outcome of a host-parasite relationship may be determined by the nutritional substrate provided by the host but emphasizes the complex relations which may exist between various nutrilites present. Whether a particular amino acid, for example, acts as a food or as an inhibitory substance may be determined by the nature and concentration of other amino acids present.

Pepsin contains a large number of amino acids which cover practically the entire range of these compounds as recorded for the banana by Steward *et al.* (1960) in their investigation of the nitrogenous compounds of this fruit. According to these authors the protein nitrogen of the pulp remains fairly constant during ripening. The majority of the individual amino acids present in the alcohol-soluble portion also remain approximately constant or decrease slightly in quantity. However, there are a few which show an increase and the order of this is shown in Table 3. Changes in the relative amounts of the nitrogen compounds also occurred between summer and winter fruit and with varying conditions of growth. A limited examination of the amino acids of the peel of the green mature Gros Michel fruit indicated that with the exception of histidine the same acids occurred as in the pulp but in differing relative amounts and a greatly reduced total nitrogen content.

TABLE 3

SELECTED AMINO-ACIDS OF THE ALCOHOL-SOLUBLE FRACTION OF CAVENDISH BANANA PULP AT THREE STAGES OF GROWTH EXPRESSED AS PERCENTAGE OF THE SOLUBLE NITROGEN RECOVERED. (From Steward *et al.* 1960)

——	Hard Green	Yellow, Green Tip	Yellow, Ripe Spots
Serine	7·3	5·0	8·1
Histidine	1·8	12·1	8·2
Arginine	8·0	10·1	9·5
Valine	2·3	3·2	5·9
Leucine	4·6	8·8	13·3

Some of these acids were used in banana treatment tests and the results appear in Table 2. None of these individual acids were particularly effective in increasing *Gloeosporium* development and it is unlikely that they are present to a limiting extent. Moreover, the changes in composition taking place as the fruit ripens are scarcely sufficient to be credited with the breaking of latency. If any effect results from these changes it must be due to an interaction between the relative amounts present rather than to the actual concentration of any individuals.

It may be that the general amino acid and protein level is too low to permit active development of the parasite under the conditions pertaining in the peel of the green fruit and it is reasonable to suppose that as ripening progresses increased energy sources and a lessened resistance to invasion enable the parasite to make more effective use of the nutritive material on hand. Where a marked acceleration has been recorded in Table 2 the increased supply of nitrogen, especially when in a highly organized form, has probably enabled the fungus to supplement its normal resources and thus proceed to earlier development.

Another explanation for the effect of increased nitrogen supply is prompted by the work of Kirkham (1954), though this is perhaps not strictly of a nutritional nature. This author showed that the reaction of *Venturia inaequalis* to phenolic

compounds can be modified by altering the composition of the basal medium with respect to its nitrogenous constituents. A high level of amino nitrogen results in a degradation of the toxic aromatic compounds by the enzyme system of the parasite. By injecting apple and pear shoots with aromatic leaf extracts and urea a similar mechanism was shown to operate *in vivo*. Although the mechanisms involved are as yet unknown, the fungal reaction seems to depend on a delicate balance between the concentration of phenolics and that of amino-acid nitrogen (Flood and Kirkham 1960). In a later section of this paper it will be shown that toxic substances or their precursors, possibly phenolic in nature, occur in the green banana. If these play any part in limiting parasitic invasion the nitrogen status of the environment could have a significant bearing on the rate of fungal advance.

IV. THE ENZYME HYPOTHESIS

(a) Introduction

The enzyme hypothesis for latent infection depends on the proposition that the fungus has insufficient enzyme potential to break down the tissues of the green fruit but that as ripening takes place the pathway for invasion is made easier by the natural softening of the tissues associated with this change. On this basis the accelerated development which takes place in the presence of a high spore load may be attributed to a co-operative effort whereby numerous small individual contributions to the enzyme pool are sufficient to make possible an earlier advance of the more vigorous individuals.

The theory that fungi and bacteria depend on enzyme secretion for their ability to break down plant tissue is an old one and many papers on the subject had already been published by the end of last century. Branfoot (1929) has reviewed this early work. It was recognised at this time that the pectic constituents of the cell wall and middle lamella form the main substrate for enzyme action and consequently the pectolytic enzymes are of particular importance in host-parasite relationships. Many fungal parasites, including *G. musarum,* maintain an intercellular existence in the early stages of invasion and only later as a more active invasion potential is built up become intracellular as well.

In 1915 Brown published the first of the series "Studies in the Physiology of Parasitism" (Brown 1915). In a lifetime devoted to this subject he and his co-workers have made many contributions to the understanding of the enzyme relationship of *Botrytis cinerea* and a number of other faculative parasites both fungi and bacteria. In America, Harter and Weimer (1921) investigated various aspects of pectolytic enzyme production in the genus *Rhizopus.*

It was early recognized that the pathogenicity of an organism is not necessarily directly related to its capacity to secrete pectolytic enzymes *in vitro,* as this

could be largely influenced by the type of media employed. The investigation of some of the apparent anomalies has shown that a particular host-parasite relationship entails a combination of factors which make it difficult to give a simple explanation on enzymatic grounds alone (Brown 1955).

Later work has taken advantage of the more advanced knowledge of the pectins and their associated enzymes and attempts have been made to characterize the particular enzymes involved and their substrates. A review of the subject including these aspects has been provided by Wood (1960).

Pectic substances are based on linear polymers of a-D-galacturonic acid units. When these are of high molecular weight they are known as pectic acids. These and their salts such as calcium and magnesium pectates are insoluble. Pectic acids which have their carboxyl groups esterified with methyl alcohol are known as pectinic acids and, when the methoxyl content is high, as pectins. The pectins are soluble and form gels with sugar and acid under suitable conditions.

In cell structure pectic substances are present in two main forms. Firstly, they are found as an important and possibly the principal component of the middle lamella, when they appear to occur in an insoluble combination with Ca and Mg and other bivalent cations. Secondly, insoluble pectic substances known collectively as protopectin occur in the matrix of the primary and to a lesser extent the secondary cell wall. These are soluble in dilute acids, yielding pectinic acids, from which they may not differ greatly except in their higher molecular weight on which their insolubility possibly depends. A thin layer of pectin may also occur where the cuticle merges into the epidermal cell wall. The importance of the pectic substances in the cell wall decreases with age as hemicellulose, lignins and other substances become deposited. In young tissue they add plasticity to the structure.

In recent years several attempts have been made to classify the pectolytic enzymes, using the particular substrate involved as a basis. Two main groups can be recognized:

(1) Polygalacturonase (PG). This enzyme splits the glycosidic bonds in the polygalacturonic acid chain, producing pectinic and pectic acids of lower molecular weight. Demain and Phaff (1957) have subdivided this group into polygalacturonase (PG), in which the preferred substrate is pectic acid or low methoxyl pectinic acids, and polymethylgalacturonase (PMG), which more actively attacks pectin.

(2) Pectinesterase (PE). This enzyme is responsible for the hydrolysis of the methyl ester groups, leaving pectinic acids of lower methoxyl content or pectic acid. Both PG and PE are commonly produced by micro-organisms. PE occurs also in the higher plants. Enzymes from different sources may differ somewhat in their properties, such as pH optimum and reaction to inhibitors.

The term protopectinase has come to be used rather loosely to cover the enzyme system responsible for solubilizing any of the insoluble pectic substances of the cell wall, including in many cases the middle lamella pectates. This enzyme has been assumed responsible for the macerating effects of many fungal extracts. Modern opinion tends to regard the postulation of a separate enzyme as unnecessary and delegates the function to PE and PG.

In spite of the attention which has been given to enzyme action in relation to pathogenesis it is still not certain what specific enzymes are involved and what is their particular substrate in the cell itself. Although it is classically assumed that pectic substances and their associated enzymes are important, attention has been paid more recently to the part played by cellulose-degrading enzymes. In addition, Kuć and Williams (1962) have shown that extracellular proteases are produced by four apple-rotting fungi, including *G. cingulata,* and suggest these may be a factor in the fungal invasion of immature fruit along with the enzymes just mentioned. The subject of pectic and cellulolytic enzymes in relation to plant disease has been well covered by Wood in the review cited above.

It is generally assumed that the softening which takes place in fruits as they ripen is due at least in part to changes in the pectic constituents of the cell walls. Carré and Horne (1927) investigated the pectic changes in apples and showed that ripening is accompanied by a breakdown in the insoluble protopectin of the cell wall, and Haller (1929) correlated the rate of conversion to the soluble form with the rate of softening of the flesh. Hansen (1938) investigated the amount of insoluble protopectin and soluble pectin in a number of fruits in the unripe state and after ripening with ethylene. In all cases ripening was accompanied by a decrease in insoluble and an increase in soluble pectic substances. This is true for the banana (von Loesecke 1949).

As a result of more recent work, Wallace, Kuć, and Draudt (1962) have suggested that the pectin in the cell wall and middle lamella of immature apple fruit is more extensively cross-linked to form a pectin-protein-metal complex resistant to hydrolysis by fungal pectolytic enzymes. As the fruit matures, the polyvalent cations of this complex are replaced by potassium and sodium with an accompanying decrease in insoluble protein. Information on the pectic transformations taking place during fruit ripening is, however, far from complete, but any form of solubilization will assist the fungal enzymes in completing the breakdown and make penetration easier.

(b) Effect of Macerating Agents on *Gloeosporium musarum* Development

Referring to Table 4, it will be seen that considerable attention has been paid to the effect of Pectinol on the parasitic activity of *G. musarum.* Pectinol 100D as used in these experiments is a commercial fungal enzyme preparation kindly made available by the Rohm & Haas Company, Philadelphia. It contains at least four pectolytic enzymes—endo–PG, exo–PG, exo–PMG (Hathway and Seakins 1959) and PE (Gothoskar *et al.* 1953). Some cellulolytic activity has also been reported by Husain and Dimond (1960).

TABLE 4

EFFECT OF VARIOUS SOLUTIONS APPLIED TO INOCULATED GREEN BANANAS ON THE DEVELOPMENT OF *Gloeosporium musarum*

(b) Pectolytic and Cell Macerating Agents

Material and Concentration	Rating	No. of Trials in Which Used	Comments
Pectinol 2% (before)	+ (1); ++ (3)	4	Strong macerating ability (1–3 hr); nil phytotoxicity
Pectinol 2%	+ (2); ++ (12)	14	
Pectinol 1%	+ (1); ++ (4)	5	ditto
Pectinol 2% (steamed 60 min) ..	+; ++	2	Nil macerating ability
Pectinol 2% (autoclaved 20 min) ..	+ (1); ++ (2)	3	ditto
Pectinol + tannic acid			See Table 8
Oxalic acid 2% (before)	+; ++	2	Slight macerating ability; highly fungitoxic
Oxalic acid 1% (before)	0 (2); + (2)	4	ditto
Oxalic acid 0·5%	0	1	Very slight macerating ability
Ascorbic acid 1·0%	+	3	Slight to moderate macerating ability; highly fungistatic
Ascorbic acid 0·5%	0; +	2	
Ascorbic acid 0·05%; 0·008% ..	0	1; 1	Nil macerating ability; nil fungitoxicity
Ammonium oxalate 1%	+	1	Nil macerating ability
Ammonium oxalate 1·5% + oxalic acid 0·4% ..	+	1	

A 1 per cent. or 2 per cent. solution of Pectinol produced a marked and consistent acceleration of invasion of the banana by *G. musarum*. This enzyme preparation was accordingly employed as a standard for comparison in the circle inoculation tests. In any one experiment it was usually amongst the best accelerators but it was not necessarily outstanding. In some cases a slight cracking occurred round the pricks following application of Pectinol solutions.

These results with Pectinol could be taken as evidence that enforced latency was due to the inability of the fungus to produce sufficient pectolytic enzymes to enable further invasion to take place under the conditions pertaining in the green fruit. However, it will be noted that acceleration was also obtained when the Pectinol solutions had been heated to destroy enzyme activity. It would seem therefore that Pectinol, at least in part, acts in a nutritive capacity, providing a substrate which enables the fungus to develop an invasion potential greater than can be developed in the fruit alone. This is comparable with the effect produced by pepsin described earlier.

Other substances known to possess some macerating effect on plant tissue were also tried. This was complicated by the marked fungitoxicity of some solutions necessitating application prior to inoculation and also to difficulty in fulfilling all the conditions required, such as raising the temperature of ammonium oxalate.

Neither oxalic acid, ascorbic acid or ammonium oxalate showed any outstanding effect on lesion development. At the higher strength the first two compounds showed a slight macerating effect when tested on potato slices but nothing approaching that displayed by Pectinol. Green (1932) carried out somewhat similar experiments with *Penicillium* on oranges and induced infection by prior application of ammonium oxalate, oxalic acid and other treatments, but in her case the treatment was probably more drastic than that employed with the banana. Ethylenediamine tetraacetic acid has been used as a macerating agent for plant tissue (Letham 1960). When the disodium salt was used in these experiments (Table 19) no disintegration of potato slices occurred with the solutions employed and the effect produced is considered to be due to physiological action rather than macerating activity.

(c) Enzyme Potential of Species of *Colletotrichum*

For support of the enzyme hypothesis it would be instructive to know whether species of *Colletotrichum* in general and *G. musarum* in particular are less effective in the production of pectolytic enzymes than other fungi not possessing the character of latency. As Wood (1960) has pointed out, the value of comparing the result of enzyme experiments carried out with several different fungi is affected by the variation which may be expected even within the one species according to the nutritional environments employed. In the experiments which follow it is therefore necessary to interpret the results with this drawback in mind.

Preliminary experiments were carried out to determine the influence of such factors as nature of the media and length of incubation time. In the case of the latter a 5-day period was decided upon as by this time enzyme production was usually well established and toxic staling products were not yet in evidence. The procedure finally adopted was as follows.

The fungi were grown on liquid media in the formulation of which a moderate rather than the maximum nutritive value was aimed at. The work of Ashour (1954) was useful in this connection. Three types of media were employed, varying only in the nitrogen constituents. The non-nitrogenous portion consisted of pectin 0·5 per cent; dextrose 0·5 per cent.; starch 2·0 per cent.; magnesium sulphate 0·05 per cent.; potassium phosphate tribasic 0·2 per cent. To this was added in the first medium asparagine, soluble casein, and peptone each 0·1 per cent.; for the second medium each form of nitrogen was used at a concentration of 0·3 per cent.; and in the third the organic nitrogen was replaced by 0·3 per cent. sodium nitrate.

For the production of active culture fluids 20 ml of the medium was added to 9-cm dia. petri dishes after seeding these with six drops of a heavy spore suspension of the fungus. In the few cases where spores were not available chopped mycelium was substituted without any apparent detriment. Four to six plates were used for each species. These were incubated for five days at 26°C. The mats from each colony were then removed to a tared filter paper, dried

and weighed for growth measurement. The liquid from the several plates was combined and used for the various tests made. These tests included pH, maceration time, pectinesterase production and lesion acceleration in the banana, using *G. musarum* as the inoculated organism in all cases.

The method of obtaining maceration time and lesion acceleration has already been described. The extent of lesion development varies somewhat with the temperatures pertaining at the time and the type of fruit used. The figures given in Tables 5 and 6 for this character are derived from the mean diameter (in mm) of the lesion extension from the inoculated circles expressed as a percentage of the mean extension shown by Pectinol treatment in the particular series under consideration.

TABLE 5

ENZYME PRODUCTION DATA FOR SIX FUNGI GROWN ON THE LOW-NITROGEN MEDIUM

Organism	No. of Times Included	Maceration (Hours to Disintegration)	Pectinesterase (Diameter (mm) of Clearing)	Lesion Acceleration (see text)	Final pH of Culture Liquid	Mean Weight of Colony (mg)
Gloeosporium musarum	6	19 (2 none)	1·8	114	7·4	167
Gloeosporium sp. (strawberry) ..	2	none	1·3	39	7·8	253
Colletotrichum gloeosporioides ..	2	21	1·5	90	7·5	215
Dothiorella gregaria	3	23	3·0	92	7·0	322
Penicillium digitatum	3	2·5	7·2	64	6·1	141
Rhizopus nigricans	3	2·1	3·3	79	4·4	144
Media only	6	none	0	46	6·1	..
Pectinol 1%	6	2·5	8·7	100	6·4	..

TABLE 6

ENZYME PRODUCTION DATA FOR SIX FUNGI GROWN ON THE HIGH-NITROGEN MEDIUM

Organism	No. of Times Included	Maceration (Hours to Disintegration)	Pectinesterase (Diameter (mm) of Clearing)	Lesion Acceleration (see text)	Final pH of Culture Fluid	Mean Weight of Colony (mg)
Gloeosporium musarum	5	7	1·2	139	8·2	210
Gloeosporium sp. (strawberry) ..	2	none	1·2	107	7·5	294
Colletotrichum gloeosporioides ..	2	22	1·2	120	7·9	229
Dothiorella gregaria	4	8	1·6	104	7·6	383
Penicillium digitatum	3	2·4	10·6	142	6·6	159
Rhizopus nigricans	3	5·5	2·8	142	7·9	142
Media only	5	none	0	99	6·1	..
Pectinol 1%	6	2·5	8·7	100	6·4	..

Pectinesterase production was determined according to the method described by McComb and McCready (1958). Three drops of the test solution were applied to 1·4-cm discs of filter paper laid on the reaction medium. Tests were made in duplicate with good correlation always between duplicates. A 1 per cent. or 2 per cent. solution of Pectinol was introduced as a check and there was little variation shown by this in the different series of experiments. The

measurement adopted for the tables was diameter of the cleared area less 1·4 cm—the diameter of the filter paper disc. There was marked difference shown in the intensity of clearing by some solutions. This is not indicated in the tables.

The origin of the fungi used was as follows:—

Gloeosporium musarum: Several different isolates from anthracnose on ripe bananas.

Gloeosporium sp. (strawberry): This designation is used in this paper for an unidentified species described by Sturgess (1957) from a ripe fruit rot of the strawberry. This species has a fairly wide host range in Queensland, where it is known to form latent infections in strawberry and papaw. It is distinct from *C. gloeosporioides*. The isolate was from strawberry.

Colletotrichum gloeosporioides: Isolated from ripe fruit spot of papaw. This organism has a wide host range in Queensland and in one or other of its forms is a common cause of ripe fruit rot.

Dothiorella gregaria: Isolated from banana fruit, where it produces a progressive shrunken rot of the green immature finger. It also causes a fruit rot of the avocado.

Penicillium digitatum: Isolated from lemon. It is the cause of green mould rot following wound infection.

Rhizopus nigricans: Isolated from papaw. It is the cause of extensive storage rot in various fruits and vegetables.

Eight series of experiments were employed, the different series being separated in some cases by considerable intervals of time. *G. musarum* was accordingly included in each experiment for reference purposes. Fungi other than those listed were employed on occasions but have not been included in the tables owing to lack of replication. The results are given in Tables 5 and 6. In a limited number of trials with the inorganic nitrogen medium the results were of the same order as with the low organic nitrogen, except that growth throughout was poorer on the inorganic.

These tables illustrate a number of points which have been noted from time to time by other workers in a similar field—for example, the ability to secrete macerating enzymes is a specific character which can be modified by the nature of the substrate. It is not necessarily related to vegetative vigour as determined by colony weight. The drift in pH also varies with the organism and the medium. There is some suggestion that low macerating activity is linked with low pectinesterase content and vice versa, but this is scarcely definite enough to point to pectinesterase being responsible in part for the maceration.

Increasing the nitrogen content resulted in an increase in the macerating ability of the culture solutions of *G. musarum* and *D. gregaria* but had little effect on the others. Pectinesterase production was in general better on the low-nitrogen medium, although the differences recorded are small. Growth in all cases except *R. nigricans* was better in the high-nitrogen medium.

From the point of view of this discussion the most interesting comparison is between the enzyme activity of the solutions and the effect of these on the development of *G. musarum* in the circle inoculation tests. Taking the low-nitrogen medium, the first four organisms listed are conspicuous for the low macerating ability of their culture liquids. In spite of this, three of them stimulated well-developed lesions in the banana, in marked contrast to *P. digitatum* and *R. nigricans,* which show much higher enzyme activity (Figure 2). This is in accordance with the many observations of lack of correlation between macerating ability and pathogenicity. The capacity of an organism to manufacture enzymes from an artificial medium is not necessarily linked with its ability to make use of the chemical substrate and energy resources existing in the tissues of a living host. In the present instance it may be significant that *C. gloeosporioides* is regarded as a close relative of *G. musarum,* while *D. gregaria* is a pathogen of the green banana.

Fig. 2.—Reaction of *Gloeosporium musarum* to solutions from fungous cultures on low organic nitrogen medium (Table 5). BO1, *G. musarum;* RO1, *Rhizopus nigricans;* XO2, media only; W3, sterile water.

On the high-nitrogen medium the differences in macerating ability are not so marked, while the lesion acceleration produced by all the culture solutions is much increased, *P. digitatum* and *R. nigricans* now attaining highest place. It will be noted that the high-nitrogen medium on its own causes marked acceleration, so the effect of the organism on the composition of the culture solution will be confused by the nutritional effect of the medium itself.

(d) Nutrition in Relation to Enzyme Potential

Reference has already been made to the effect of heating a Pectinol solution in respect to its accelerating action in the circle inoculation tests. The effect of heat on culture solutions of three of the organisms use in the enzyme experiments was also investigated. Samples of these solutions were heated in a steamer for 60 min, when potato slice tests showed that all macerating enzymes had been destroyed. The heated and unheated solutions were then applied to bananas in standard circle inoculation tests. From Table 7 it will be seen that with *G. musarum* and *P. digitatum* there was little difference in the accelerating activity of the heated and unheated solutions. It would therefore appear that this activity is dependent on the extent to which the solution provides a suitable substrate for *G. musarum* to organize its own enzyme complement rather than on the already present content of macerating enzymes.

TABLE 7

EFFECT OF UNHEATED AND HEATED CULTURE SOLUTIONS ON *Gloeosporium musarum* DEVELOPMENT IN THE GREEN BANANA

Culture Solution	Number of Trials on Low or High Nitrogen Media	Mean Lesion Development (mm)	
		Unheated	Heated
Gloeosporium musarum ..	1 low; 2 high	6·9	6·2
Penicillium digitatum ..	1 low; 1 high	10·8	10·1
Dothiorella gregaria ..	1 low; 1 high	6·3	1·2
Pectinol	1 (1%); 2 (2%)	11·6	10·3

It might be stated here that some of the culture solutions used in these enzyme experiments were amongst the best accelerators of anthracnose development of all the substances tried. These were among the few materials which enabled well-marked development to take place while the fruit was still in the hard green stage.

Under the conditions of these experiments the three species of *Gloeosporium* showed up as poor sources of macerating and pectinesterase enzymes. However, it is shown by comparison with *P. digitatum* and *R. nigricans* that factors other than the presence of these enzymes in the culture solution must be involved in stimulating the development of *G. musarum* in the banana. Either macerating and pectinesterase activity *in vitro* does not disclose all the enzyme potential responsible for fungal advance or some factor other than enzyme activity of the solution is of overriding importance.

It is postulated that this essential factor is a suitable basic substrate from which the fungus can organize as required and at the correct site for effective use its own specialized enzyme complement adapted to the particular host-parasite combination concerned. The substrate necessary would conceivably vary with the particular organism and host tissue involved. This hypothesis fits in

with the results of the nutritional trials summarized in Table 2, with the effect of the high-nitrogen medium in the enzyme experiments, and with the failure of heat to eliminate the lesion-accelerating properties of Pectinol and some fungal culture solutions.

This proposition does not invalidate the hypothesis that latency is due to the inability of *Gloeosporium* to secrete sufficient pectolytic enzymes to enable it to penetrate the green fruit, but rather shifts the emphasis towards the general metabolic state of the fungus in relation to its substrate and energy resources. The position is summed up by Lichstein (1960) when he states that the biosynthetic pathways of a micro-organism are influenced by the nutritional environment, which may permit or preclude the development of metabolic patterns with which the cell is genetically endowed.

V. THE TOXIN HYPOTHESIS

(a) Introduction

The existence of toxins in plant tissue has been widely postulated as a basis for host resistance to parasitic fungi. There has been a tendency in recent years to distinguish between a toxic substance already present in the host before invasion occurs (a preformed toxin) and a toxin which is produced as a result of a specific interaction between the metabolic systems of host and parasite (a phytoalexin).

Preformed toxins which have been implicated in disease resistance are commonly related to the tannins. Polyphenolic substances are common constituents of plant tissue although their exact function there is still a matter for argument. Byrde (1957) has pointed out that the simple tannins such as chlorogenic acid, the quinones formed from these by the action of polyphenoloxidase, and the final polymerised products, the coloured phlobaphenes, have each been associated with resistance factors by one or more investigators. With these phenolic compounds it is necessary to consider, in addition to direct toxic action, an inhibitory effect on fungal-macerating enzymes.

The polyphenoloxidase systems of host and parasite are intimately bound up with the various changes taking place and may be involved in promoting susceptibility or resistance, depending at which end of the chain of reaction the toxic substance lies.

Newton and Anderson (1929) pointed out earlier that phenolic substances could not very well exist free in the plant in a toxic form and any effect they have on resistance may be due to a reaction with the metabolites of the parasite, the complexity of which could account for varying degrees of susceptibility. Approaching the subject from the cytological angle, Dufrénoy (1936) also stressed the importance of the host-parasite interaction. There is now an increasing opinion that it is not the actual level of a phenolic substance which is the important factor, but the metabolic changes in which the phenols are involved (Allen 1959*a*).

With these views in mind, failure to obtain evidence of a toxin in unparasitized tissue need not necessarily mean that one is not active when the tissue becomes invaded and host-parasite interactions come into play. Furthermore, a toxic substance may exist preformed in one part of the host and in another may be produced in response to fungal attack (Kuć 1955). In these cases it is necessary to assume the existence of a toxin precursor, a situation which may bridge to some extent the current distinction between preformed toxins and phytoalexins.

(b) Effect of Tannins on *Gloeosporium musarum* Development

The influence of tannic acid on the development of *G. musarum* was investigated in the circle inoculation tests (Table 8). The effect was nil or if anything suppressive. This may have been due to toxicity and/or to the effect of tannic acid on enzyme activity. The latter is well illustrated by the figures for the combinations of various strengths of tannic acid and 2 per cent. Pectinol (see also Figure 3).

TABLE 8

EFFECT OF VARIOUS SOLUTIONS APPLIED TO INOCULATED GREEN BANANAS ON ANTHRACNOSE DEVELOPMENT

(c) Tannin and Associated Chemicals

Material and Concentration	Rating	No. of Trials in Which Used	Comments
Tannic acid 0·5%	− (1); 0 (4)	5	Highly fungitoxic
Tannic acid 0·1%	0	2	Fungitoxic
Tannic acid 0·05%	0	2	Slightly fungistatic
Tannic acid 1%—Pectinol 2·0%	0	2	20 hours to disintegration
Tannic acid 0·5 %—Pectinol 2·0%	+	1	17 + hours to disintegration
Tannic acid 0·25%—Pectinol 2·0%	+	1	17 hours to disintegration
Tannic acid 0·1 %—Pectinol 2·0%	+/++	1	3·5 hours to disintegration
Tannic acid 0·05%—Pectinol 2·0%	++	1	3 hours to disintegration
Ferric chloride 0·5%	++	1	Slight macerating ability; and highly fungitoxic
Ferric chloride 0·25%	+; ++	2	Slight macerating ability; and highly fungitoxic
Ferric chloride 0·1%	+	1	Slight macerating ability; and highly fungitoxic
Ferrous sulphate 0·5%	++	1	Slight macerating ability; and highly fungitoxic
Ferrous sulphate 0·2%	+; ++	2	Slight macerating ability; and highly fungitoxic
Ferrous sulphate 0·1%	0/+	1	Slight macerating ability; and highly fungitoxic
Glutathione 1·0%; 0·5%	0	2; 1	
a-naphthylthiourea 0·05% ..	−; 0	2	
Thiourea 0·1%; 0·076%	0	2; 1	
Urea 0·5%	0	3	
Sodium diethyldithiocarbamate 0·2%; 0·002%	0	1; 1	

Ferric chloride and ferrous sulphate are included with tannic acid since they were used with the object of removing some of the phenolic compounds which might serve as a source of antifungal toxin. On the results obtained such a reaction would seem to be indicated, but it must be kept in mind that other physiological effects may be involved. For example, both these chemicals have a mild macerating action.

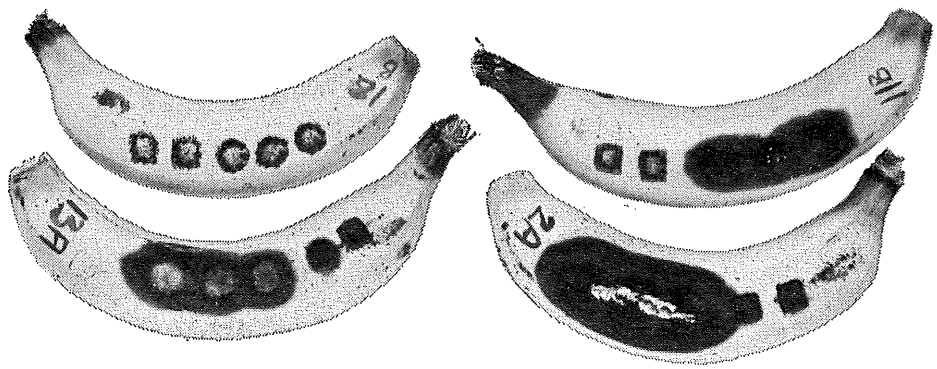

Fig. 3.—Reaction of *Gloeosporium musarum* to certain chemicals listed in Table 8. 18B, water only; 13A, ferric chloride 0·25%; 11B, Pectinol 2%, tannic acid 0·5%; 2A, Pectinol 2%.

If a resistance-inducing toxin is brought into action by the oxidation of a polyphenolic precursor, then the inactivation of polyphenoloxidase should result in a reduction in the amount of toxic substance, thus allowing invasion to proceed. Such an effect has apparently been obtained by Byrde (1957), who showed that treatment of injured tissue of an apple variety resistant to *S. fructigena* with 0·062 M glutathione reduced resistance to spore infection. Gothoskar *et al.* (1955) refer to the inhibition of ascorbic acid oxidase and polyphenol oxidase by sodium diethyldithiocarbamate and demonstrate that treatment of Fusarium-resistant tomato cuttings with a 10^{-3} and 10^{-4} M solution would increase wilting and vascular browning.

Five compounds—glutathione, sodium diethyldithiocarbamate, a-naphthylthiourea, thiourea and urea—reputed to inactivate polyphenoloxidase were used in the banana inoculation tests. No evidence was obtained of any effect on invasion by *G. musarum*. This could be taken as an indication that an oxidized polyphenol was not responsible for the resistance of the green banana fruit. This will be referred to again later in this section.

(c) Examination for the Presence of a Phytoalexin

The term phytoalexin was first used by Müller and Börger (1940) to cover an antibiotic substance formed as a specific response to the interaction of metabolites of a host plant and a parasite invading it. Later Müller (1958) developed a simple and effective method of further investigating the subject by using the sterile inner tissue of French bean pods. He considered phytoalexins to

be at least one of the factors responsible for the hypersensitive reaction leading to host resistance. A somewhat similar development was obtained by Kuć (1955) in potato and corn tissue inoculated with *Helminthosporium carbonum* and other fungi. This type of reaction has since received the attention of other workers and in several instances a specific toxic substance has been isolated and identified.

The amount of phytoalexin produced by the host in response to fungal invasion varies with, amongst other factors, the age of the tissue (Müller 1958). The formation of a substance of the phytoalexin type in the green but not the ripe banana would form a neat explanation of latent infection but would lead to the need for further explanations. For example, there is no definite hypersensitive reaction although discolouration of the cell wall adjacent to the hyphal knot is sometimes a feature of the latent stage (Simmonds 1941). A more pronounced reaction in the case of the tomato has been recorded by Illman *et al.* (1959). Moreover, the balance between fungus and anti-fungal substance would need to be of a very sensitive nature, for at least some and often many of the infections originally rendered stationary resume normal development later when, presumably, the metabolic changes in the ripening fruit would render the hypersensitive reaction no longer operative.

Several attempts were made to obtain evidence for the production of a host-parasite induced toxin by methods adapted from those devised by Müller. Circular areas on the green banana skin were inoculated with spores of *Gloeosporium musarum* in water suspension. After allowing 24 hr or more for the production of appressoria and the establishment of latent infections, the residue of original spores was removed by washing and a fresh drop of water was applied to the inoculated area. After approximately 24 or 48 hr, portions of these drops were removed and tested against *G. musarum* for inhibitory action on spore germination. Drops taken from uninoculated circles were examined for comparison.

In most of these experiments there was no essential difference between growth in diffusates from the inoculated or uninoculated surface, although the characteristic phytoalexin production was readily demonstrated when *G. musarum* or *C. gloeosporioides* was applied to bean or pea pods. In the few instances where growth was less in diffusates from the inoculated surface the difference was of a low order.

Failure to obtain positive evidence for phytoalexin formation could be due to the thickness of the cuticle on the banana fruit preventing the diffusion of toxic substances through to the drop of water on the surface. Attempts were made to overcome this by the use of minute pricks, rubbing the surface, and wiping lightly with chloroform before inoculation, but the results failed to supply satisfactory evidence of a phytoalexin reaction. A typical example of the figures obtained is given in Table 9. Examination showed appressoria present to the extent of 240 to the sq. mm. on the pricked inoculated skin.

TABLE 9

COMPARISON OF GERM-TUBE DEVELOPMENT (IN μ) OF *Gloeosporium musarum* IN DIFFUSATES FROM INOCULATED AND UNINOCULATED BANANA SKIN

Treatment	Inoculated	Uninoculated
Skin uninjured	125	129
Minute pricks	165	164
Surface rubbed	141	165
Wiped with chloroform	148	181
s.e.	24·5	

Growth in sterile water (no fruit contact)—134 μ

An attempt was made also to extract a toxic material from banana peel, using the method described by Kuć, Ullstrup, and Quackenbush (1955). Both ethyl and methyl alcohol were used as extractants. No essential difference was obtained in the growth of *G. musarum* and other fungi on media including the extracts from inoculated or uninoculated fruit.

(d) Examination for a Preformed Toxin in Cool Extracts

In spite of the amount of work which has been done on the occurrence of toxins in plant tissue there are comparatively few instances where a definite relationship between a preformed toxin and host resistance has been proved. It is far from easy to show that a suspected toxic substance is in fact present in adequate concentration at the site of invasion. In most cases reliance has to be placed on evidence of a circumstantial nature. Moreover, the specificity so characteristic of parasitism seems to need a more intimate host-parasite relationship than is offered by a ready-made toxin.

The classical example of a preformed toxin is found in the resistance of onions to *Colletotrichum circinans* due to protocatechuic acid and catechol located in the brown outer scales of coloured onions (Link, Angell, and Walker 1929). As an extension to this work Walker and Link (1935) examined the effect of 21 phenolic compounds on four fungi and found a great diversity in their toxic properties. As a result these authors stress the need to ascertain the specific concentration at which the suspected toxin occurs in the plant and the toxic or stimulative effect it has on the parasite at this concentration before regarding it as a disease-resistance mechanism.

A few other reasonably well based claims for the existence of toxic substances have been made from time to time. For example, solanine extracted from green but not ripe tomato is toxic to *Colletotrichum phomoides* at a concentration of 500 p.p.m. and Allison (1951) considers that this substance may account, at least in part, for the failure of the fungus to colonize green fruit after invasion has occurred. Kirkum (1954) was able to increase the resistance of apple and pear shoots to *Venturia inaequalis* and *V. pirina* by injecting phenolic extracts from resistant varieties.

To return to the banana, Cook and Taubenhaus (1911) investigated the effect of various concentrations of tannin on a number of species of *Gloeosporium,* including *G. musarum.* In most cases concentrations above 0·1 per cent. caused a marked reduction in growth. A lower concentration may be sufficient to prevent germination and a higher concentration may be tolerated when the fungus is grown in a suitable media.

Barnell and Barnell (1945) describe the location of tannins within the Gros Michel banana fruit and the changes which take place during ripening. The tannin occurs in a system of latex vessels associated with vascular bundles and also scattered parenchymatous cells in the outer and middle region of the peel. During ripening the contents of the latex vessels dry out and the tannin disappears but some may appear in adjacent cells. On the other hand, the contents of the small scattered tannin cells appears to undergo little change. These authors consider that as anthracnose spotting does not appear until tannin concentration is low it is reasonable to suggest the existence of a causal relationship between the tannin concentration of the peel and the activity of these infections. They also discuss the inhibitory action of water extracts of the banana on taka diastase and point out that an oxidized extract of banana pulp has less inhibitory action than the unoxidized.

The location of tannin substances within the skin of the Cavendish banana appears to follow the same pattern as described by Barnell and Barnell for the Gros Michel. The restriction imposed on the sphere of influence of the tannin while it is contained within the system of latex vessels is an important argument against a toxic action arising from this source.

Chakraverty (1957) made germination counts of *G. musarum* in juice from the skin of green and ripe banana fruit with and without the addition of 2 per cent. malt or distilled water and interpreted the results as indicating the presence of an inhibitory substance in the undiluted sap of green fruit. On the figures given, such a substance, if present, must be of a fairly low order of activity.

The effect on spore development of extracts of the peel of green and ripe Cavendish bananas has been investigated here. Spores were also germinated in drops into which had been inserted thin slices taken from the upper layers of the skin. From both extracts and slices there was no consistent evidence of the presence of a toxin—at least not one with a potency which could be expected to give it pathological significance. As would be expected, growth was in general better using ripe than green fruit, although the latter provided a better medium than water alone. Spores immediately alongside tissue pieces sometimes showed poorer growth than those situated further away and the presence of latex also reduced growth in some experiments. A typical example of the results obtained from these germination studies is contained in Table 10. Examination of cool extracts thus lends little support to the preformed toxin theory. Hot extracts, to be discussed later, gave an unexpectedly different picture.

TABLE 10

EFFECT OF A COOL AQUEOUS EXTRACT OF GREEN BANANA PEEL ON THE GERM-TUBE DEVELOPMENT OF *Gloeosporium musarum*

Germinating Medium	Average Germ-tube Length (in μ) of 20 Spores Measured in Each of 4 Replicates				Mean
Green extract	119	122	105	109	114
Green extract + nutrient*	123	119	126	159	132
Sterile water	76	66	74	69	71
Sterile water + nutrient*	156	152	159	193	165

s.e. Means 6·52. Necessary differences for significance, 20·9 (5%); 29·9 (1%)

* One loop of standard potato-dextrose nutrient solution added to each double drop of extract or water to achieve a more uniform germination and growth in the water control

(e) Evidence for an Enzyme Inactivator in Cool Extracts

It could be that phenolic substances in the banana act as enzyme inhibitors rather than as distinct toxins as was suggested by Barnell and Barnell (1945). In more recent times and working mainly with pome fruits, several investigators have reported the inactivation of pectolytic enzymes by extracts containing phenolic substances or by solutions of tannins. As a further refinement, Byrde, Fielding, and Williams (1960) investigated the action of specific polyphenols on the macerating and polygalacturonase activity of *Sclerotinia fructigena* and came to the conclusion that the polyphenols in apple fruit probably form a defensive mechanism against brown rot not by a direct toxic action which cannot be demonstrated but through their ability to inactivate the extracellular enzymes of the fungus. On injury to the fruit the polyphenolic compounds are oxidized by host enzymes and in the oxidized state are then able to exert their enzyme-inactivating effect.

The effect of water extracts of green and ripe banana peel on the macerating action of Pectinol solutions was investigated. Under aseptic conditions 25 g of peel was finely sliced and added to 100 ml of distilled water and allowed to stand for 20 hr in a closed flask. Then 2 ml of an acetate buffer of pH 4·5 was added to 20-ml portions of the liquid extract followed by 10 ml of a 1·5 per cent. Pectinol solution. Alteration in the activity of the Pectinol was noted by the potato slice technique. The result of one such experiment involving four replications each of which yielded identical figures is given in Table 11.

TABLE 11

EFFECT OF EXTRACTS OF GREEN AND RIPE BANANA PEEL ON THE MACERATING ENZYMES OF A PECTINOL SOLUTION

Extract	pH of Solution	Hours to Maceration Point
Green skin ..	4·8	Nil maceration in 24 hr.
Ripe skin	4·9	1
Water check ..	4·5	0·5

In a second experiment the results were similar but the effect less marked. Both these experiments were carried out in March 1962, but when the work was repeated later in the year it was not possible to reproduce the results. However, a similar experiment carried out in February of the following year again demonstrated the same positive inactivation.

The production of an excessive amount of latex by fruit harvested in midsummer may explain the activity of extracts from the peel of green fruit obtained at this time of the year. This supposition obtains support from the fact that a mixture of extruded latex and water to form a moderately cloudy suspension will inactivate Pectinol solutions in the same way as the green skin extract.

The restriction of this activity to one time of the year—incidentally the period during which anthracnose is most troublesome—argues against its acceptance as a cause of latency. Moreover, the latex is closely confined in the system of latex vessels which are situated some little distance below the surface and away from the site of any possible action.

(f) Evidence for a Toxin in Hot Extracts

While the examination of cold extracts of banana peel yielded no information which could usefully be employed in explaining latent infection, the position changed when hot extracts were used and an interesting field for speculation opened up.

Briefly, if thin slices of the outer peel of the green banana are immersed in boiling water and allowed to cool, an extract is obtained which may completely inhibit spore development. This did not occur when the skin of a ripe fruit was employed or when the slices were boiled for two minutes before cooling. Toxic activity is still present if water at 80°C is used, but on lowering the temperature of immersion to 60°C the effect is lost.

A general picture of the position may be obtained by examining Table 12. This table gives the mean germ-tube development obtained from a series of extractions carried out in six replications each at a separate time with a different batch of fruit. As germ-tube growth varied in each replication with temperature and time of incubation, the figures for this growth have been expressed as percentage of the growth occurring in standard nutrient. This latter consisted of one loopful of standard potato-dextrose solution (potato 20 per cent., dextrose 2 per cent.) added to a double drop of sterile distilled water. The average growth in this nutrient at the time of measurement was 0·34 mm. The means given in the table were obtained from 40 measurements comprising 20 spores from each of two germination drops.

TABLE 12

GERM-TUBE DEVELOPMENT IN VARIOUS EXTRACTS OF THE OUTER BANANA PEEL EXPRESSED AS PERCENTAGE OF GROWTH IN STANDARD NUTRIENT

Extract	31.vii.62	23.viii.62	20.ix.62	18.x.62	1.xi.62	11.vi.63	Mean
Green cool ..	103	111	112	114	105	105	108
Ripe cool ..	99	101	130	106	115	84	106
Green hot ..	0	0·1	2	0·5	27	0·2	5
Ripe hot ..	73	79	111	92	92	91	90
Green boiled	95	99	113	104	103	101	103
Ripe boiled ..	91	102	109	98	100	84	97

s.e. Means 2·87. Necessary differences for significance, 11·6 (1%), 8.5 (5%) (Green hot excluded from analysis)

For the extractions the following procedure has been adopted. The outer skin of the fruit is thinly pared off with a stainless steel knife. An average Cavendish banana yields about 8 g of peelings, which represents approximately 20 per cent. of the total weight of the peel. The peelings are cut transversely into small pieces and immersed into a beaker of sterile distilled water at the appropriate temperature, using 4 ml of water for every gram of tissue. The extract is allowed to stand for one to three hours before sampling. The exact time is apparently not important but it is desirable to stir the tissue pieces occasionally during this period.

For a "cool" extract the water is used at room temperature. For a "hot" extract the water is brought to the boil and then allowed to cool to 95°C. The tissue pieces are then quickly immersed, when the temperature falls almost immediately to the vicinity of 80°C. For the "boiled" extract the peelings are immersed in boiling water and the water boiled for a period of two minutes.

If extraction is carried out during the warmer months with room temperatures approaching 27°C., the toxic property of the hot extract may be relatively low unless the extraction and spore germination are carried out at a lower temperature. During the warmer weather therefore the extracting material was removed to a temperature between 10° and 20°C as soon as the temperature had fallen to 60°C, and spore germination was carried out at approximately 20°C.

The investigation of the nature of this toxic reaction has not proceeded sufficiently far for the final picture of the position to be given but it is pertinent to the subject under discussion to review the results so far obtained.

Because phenolic compounds are commonly implicated in preformed toxins, the extracts were subjected to the ferric chloride and nitroso tests (Reeve 1951) for catechol derivatives and the starch-iodide test for quinones. Since polyphenoloxidase plays an important part in the phenolic reactions of the plant, a test for this enzyme was carried out with guaiacum and a note made of any darkening of the tissue pieces in the upper layers of the extract suggesting oxidation of phenolic compounds. The results of a number of these tests are summarized in Table 13.

TABLE 13

RESULTS OF SELECTED CHEMICAL TESTS ON VARIOUS EXTRACTS OF GREEN AND RIPE BANANA SKIN

Extract	Nitroso	Ferric Chloride	Quinone	Guaiacum	Tissue Browning
Green cool ..	—	—	?	++	—
Ripe cool ..	Slight	—or a-typical	?	++	+
Green hot ..	+	+	+	+	+
Ripe hot ..	+	+	?	+	+
Green boiled ..	+	+	—	—	—
Ripe boiled ..	+	+	—	—	—

In the green hot extract a catechol compound or compounds are present together with a polyphenoloxidase system capable of effecting oxidation of these compounds, and this evidently takes place. The ripe hot extract appears similar unless a negative quinone test is assumed, suggesting that the substrate or course of oxidation is different. In both the green and the ripe boiled extracts a catechol substrate is present but the enzymes have been destroyed.

The cool extracts are less easy to interpret. No reaction for catechol or its derivative is given by the cool green extract. This could be a question of solubility or inaccessibility, and if the slices are crushed before extracting a positive though less intense reaction for catechol compounds is obtained and the extract assumes slight toxic properties. Maceration in a Waring blender led to a more pronounced but somewhat atypical nitroso reaction and increased toxicity of the extract (Table 14), but this was not as marked as might be expected if poor mechanical liberation was the sole cause of low toxicity. The possible presence of an inactivating system cannot be ignored. The impression given by the reactions of the ripe cool extract is that there have been changes in the tannin constituents during ripening. This may refer mainly to the contents of the latex vessels as suggested by Barnell and Barnell (1945).

TABLE 14

EFFECT OF METHOD OF PREPARATION ON THE PRODUCTION OF TOXIN BY BANANA SKIN IN COOL EXTRACTS

Preparation of Tissue	Germ-tube Development as Percentage Growth in Standard Nutrient
Experiment A	
Normal slice, cool extract ..	101
Crushed slice, cool extract ..	77
Normal hot extract	0
Experiment B	
Normal slice, cool extract ..	90
Macerated slice, cool extract	60
Normal hot extract	0

Darkening of the slices due to oxidation from the air during the course of the extraction accompanies toxin formation. However, if this is prevented by covering the extracting material with liquid paraffin, the production of toxin still occurs and is evidently not linked to this particular oxidation reaction. The pH of both green and ripe hot extracts is lower than that of the others.

Further information is obtained by comparing the toxic reaction of combinations of various extracts. These combinations were made by taking one drop of each extract and mixing to form the usual double drop to which spores are added for testing. The results from three series are given in Table 15. For unknown reasons results are not always consistent for these particular reactions.

TABLE 15

EFFECT ON TOXICITY OF COMBINING VARIOUS EXTRACTS OF BANANA SKIN
Germ-tube Development of *Gloeosporium musarum* Expressed as Percentage Growth in Standard Nutrient

Extract Combination	23.viii.62	20.ix.62	11.vi.63	Mean
Green hot : extract only ..	0·1	2	0·2	0·8
Green hot : water	2	26	3	10
Green hot : green cool ..	3	3	6	4
Green hot : ripe cool ..	87	58	85	77
Green boiled : green cool	32	5	5	14
Green boiled : ripe cool ..	111	104	85	100

The addition of water to a green hot extract lowers the toxicity to an extent varying from one batch of fruit to another. Green cool extract also acts as a dilutent. The addition of ripe cool to green hot extract always results in a marked reduction in toxicity and the ripe fruit evidently contains a substance capable of inactivating the toxin. The combination of green cool and green boiled extracts both of which are non-toxic may produce a highly toxic solution. The combination ripe cool and green boiled is without effect in this way.

Certain deductions appear permissible from these findings. Firstly, the enzyme complement necessary for the production of the toxin is present in the green fruit but the essential substrate is either inaccessible or rendered inactive by the presence of an inhibitor. In the green hot and green boiled extract these barriers to the activity of the substrate are removed. In the green boiled extract the necessary enzymes are removed as well. Secondly, if toxic substances are capable of being produced in the ripe fruit—and the differences in growth potential between the ripe cool and ripe hot extracts suggest this is at least possible to a modified degree—then the presence of an inhibitory substance will counteract their effect and enable the fungus to develop.

The question of the identity of the toxic substance arises and it is proposed to investigate this aspect further. The main catechol compound or compounds giving rise to the strongly positive nitroso and ferric chloride reactions is not the one directly responsible for toxicity. Oxidation by polyphenoloxidase may be involved but it will be remembered that the application of polyphenoloxidase inhibitors in the banana inoculation tests (Table 8) gave no evidence of this. Palmer (1961) states that the browning of banana fruits is due to the oxidation of 3-hydroxytyramine which is synthesized through tyramine from tyrosine (Buckley 1961). This could be the basis for the synthesis of allied toxic compounds.

Some circumstantial evidence is available linking the toxin with latent infection. For example, the part of the peel responsible for the production of the toxin appears to lie in the outer layers, as shown in Table 16. The slices used in (1) and (5) were typical of those used in the extracts already described. They comprised about one-fifth of the total peel weight and included many of the small tannin cells scattered through the outer parenchymatous tissue but excluded the majority of the large latex vessels associated with the vascular bundles.

TABLE 16

GERM-TUBE DEVELOPMENT OF *Gloeosporium musarum* IN HOT EXTRACTS OF DIFFERENT PARTS OF THE GREEN BANANA PEEL

Portion of Peel Extracted (1 g tissue in 4 ml water)	Development Expressed as Percentage Growth in Standard Nutrient
1. Thin slice from outside	0
2. Thicker slice from outside	10
3. Thick slice after removing (1)	73
4. Whole skin	90
5. As (1), second experiment	0
6. As (3), second experiment	70

A marked difference has been noted in the amount of toxin produced by different types or batches of fruit. This difference can sometimes be correlated with differences in susceptibility to *Gloeosporium* infection. In general it is the "hard" fruit of moderate size and fullness from older plantations where growth is less lush that produce consistently high levels of toxin. Fruit which have been scalded by cold and sun and large, very full fruit from young plantations often show evidence of low toxin potential. Some of these differences are shown in Table 17. In assessing susceptibility to anthracnose, inoculation took the form of a spore suspension in water of *G. musarum* applied to from four to six circles on the uninjured surface of the banana fruit. Whether the apparent correlation between toxic reaction and susceptibility is a direct or associated effect remains to be determined.

TABLE 17

EFFECT OF TYPE OF FRUIT ON TOXIN PRODUCTION AND ANTHRACNOSE DEVELOPMENT

Date of Trial	Type of Fruit	Growth in Green Hot Extract as Percentage Growth in Standard Nutrient	Mean Lesion Diameter on Inoculated Fruit (mm)
26.vii.62	Large and full	19·3	15·5 (2 fruit)
	Small and thin	0	3·7 (2 fruit)
9.viii.62	Medium size and fullness—scalded	77·8	8·5 (3 fruits)
	Medium size and fullness—unscalded	0	Trace (3 fruit)
30.viii.62	Very large and very full ..	41·7	7·1 (4 fruit)
	Scalded, medium full ..	7·2	7·7 (4 fruit)
	Normal, medium full ..	4·5	2·7 (4 fruit)
	Small, medium full	0·02	0·3 (4 fruit)

Latent infection is not restricted to the banana, so if a toxin is at the basis of the phenomenon it should be possible to demonstrate its presence in other fruit subject to *Gloeosporium* attack. Some exploratory work has been done in this connection and the results can be summarized as follows.

The mango is similar to the banana in having a fairly well marked ripening phase and in the simple chemical tests employed the reactions resembled those of the latter although different tannin compounds may be involved. Polyphenoloxidase is present except in the boiled extract, but there is no tissue browning. The green hot extract has a marked effect on germ-tube development as with the banana, but contrary to the latter this effect is also found in the ripe hot and the boiled extracts (Table 18).

TABLE 18

EFFECT OF SKIN EXTRACTS OF VARIOUS FRUIT ON THE GERM-TUBE DEVELOPMENT OF SPECIES OF *Gloeosporium*

Growth Expressed as Percentage Growth in Standard Nutrient

Fruit	Test Organism	No. of Trials	Green Cool	Green Hot	Green Boiled	Ripe Cool	Ripe Hot	Ripe Boiled
Banana ..	(From Table 12)	6	108	5	103	106	90	97
Mango ..	*Colletotrichum gloeosporioides*							
	(*a*) (papaw strain)	2	97·5	0·3	0·2	61·1	0·2	0·1
	(*b*) (mango strain)	2	102·1	0·4	0·1	29·1	0·02	0·02
Avocado ..	*Colletotrichum gloeosporioides* (papaw strain)	4	87·0	16·9	28·6	57·2	39·7	39·0
Strawberry ..	*Gloeosporium* sp. .. .. (strawberry)	3	16·8	6·9	1·9	24·3	25·4	27·6
Papaw ..	*Colletotrichum gloeosporioides* (papaw strain)	5	71·1	91·7	90·1	77·8	81·1	81·7

The avocado has a less sharply defined ripening phase than the banana and the mango. Catechol compounds are present but polyphenoloxidase is very little in evidence in the hot extracts. Growth in the green hot extract was always less than in the other extracts but complete suppression of growth was not so frequent as in the banana. Growth in green cool extract was unaffected, but in contrast with the banana growth in the remaining extracts showed some reduction.

Strawberries were the only non-climacteric type of fruit examined. They give a positive reaction for catechol compounds, though this is less intense than in the banana and avocado. Polyphenoloxidase according to the guaiacum test is absent or in low supply. None of the extracts approached the standard nutrient in suitability for growth and the main difference appeared to be between the green extracts as a whole and the ripe. Growth was much less in the former.

In one trial with custard apple (*Annona squamosa*) all extracts including even the green cool proved highly toxic. *Gloeosporium* infection is rarely seen on this fruit.

The papaw presents a marked difference in many respects to the other fruit dealt with. The speed of ripening varies with the variety and may be a fairly gradual process. There is apparently little tannin material present even in the green fruit. Polyphenoloxidase is present but tissue browning does not occur. There is no evidence for the presence of a toxic substance in any of the extracts. The papaw therefore provides an exception not easily dismissed in any attempt to find a common basis for an explanation of latent infection in terms of the toxic reaction of the host.

If the presence of a toxin is to be accepted as an explanation of latency, it seems necessary to assume that the fungus in attempting to invade the green fruit comes in contact with a toxin closely bound up with the tissues of the plant or permits the production of toxic substances by removing an inactivating system in the course of its own metabolic activity. As is usual in these cases, evidence for a toxin hypothesis is largely circumstantial. In favour is the disappearance or inhibition of the toxin as the fruit ripens and the linking of degree of toxicity with rate of invasion of the fungus once latency is broken. On the other hand, a considerable variation in the degree of toxicity as indicated by the methods employed here does occur and it might be expected that fruit possessing a low value would be suitable for some invasion by *Gloeosporium* while the fruit is still green. This does not occur under natural conditions. The rapid development which occurs when *G. musarum* is inoculated to the exposed pulp of a green banana is readily interpreted as a wound reaction but it could be that toxic substances have been removed with the peel.

VI. THE RESPIRATION HYPOTHESIS

An attempt will be made in this section to explain the effect of fruit ripening on latent infection. Two other well-known conditions leading to the breaking of latency, namely wounding and methyl bromide fumigation, will also be discussed.

(a) Metabolic Pattern of Ripening Fruit in Relation to Parasitism

The outstanding importance of fruit maturity in determining when latent infections will become active has already been stressed. Wardlaw and Leonard (1936) observe that the appearance of *Gloeosporium* infections, whether on the banana, papaw, avocado or mango, occurs at a certain stage in the ripening process, usually when the fruit is passing from the eating ripe stage to the later phases of senescence. They state "the development of latent *Gloeosporium* infections is as much an indication of physiological state as are other non-pathological features such as the development of skin-colour or the commencement of softening."

There are three major changes associated with the ripening process in fruits: (1) an increase in soluble pectic substances leading to a freeing of the cells along the line of the middle lamella with consequent softening, (2) a conversion of reserve material in the form of polysaccharides to simple sugars; and (3) changes in the nature of the respiratory gas exchange. The first two of these have received mention in previous sections, leaving the respiratory change to be discussed at this stage.

In most varieties of fruits subject to latent infections, including banana, mango, papaw and avocado, there is a well-defined climacteric rise in the respiratory rate as maturity approaches. This has been investigated in detail for the Gros Michel banana by Wardlaw and Leonard (1940). In the pre-climacteric, firm, green stage the respiration rate is low and approximately constant. The onset of ripening is heralded by the climacteric phase lasting 24-48 hr. During this period the respiration rate rises sharply to a peak of about four times the normal. It is about this time that "springing" or softening of the fruit commences, followed by colour changes in the skin. After reaching the climacteric peak the respiration rate gradually falls as the fruit passes through the various stages of ripeness, but does not approach the original low value until the fruit is well overripe.

The exact form of the climacteric rise and the time taken vary with different species of fruit but the common characteristic is a marked increase in metabolic activity. In the Cavendish banana under natural conditions anthracnose spotting does not usually appear until the fruit has reached the full yellow stage, but with artificial inoculation, especially if this is relatively heavy, earlier appearance may be expected, though not before the fruit are in the "springing" condition. Similarly, in the case of the papaw (Wardlaw and Leonard 1935) and the mango (Wardlaw and Leonard 1936), anthracnose does not appear until the climacteric phase has been reached and usually reaches its maximum visible development subsequent to the climacteric peak.

Sitterly and Shay (1960) found that active development of four apple-rotting fungi, including *Glomerella cingulata,* commenced at the time of the climacteric respiratory increase whether this occurred at the normal time or was brought forward several weeks by the action of maleic hydrazide or ethylene.

In contrast with this type of fruit there are others classed as non-climacteric in which there is a gradual decline in respiration from the immature to the mature stages without any change in the pattern to mark the transition from the former

to the latter. Citrus come within this class and it may be significant that these fruit are not usually subject to *Gloeosporium* ripe fruit rot until in a senescent stage following long storage. In describing the effect of black spot (*Guignardia citricarpa*) on citrus, Kiely (1948) states that as the rind of the fruit approaches maturity the mycelium arising fom latent infections is progressively more capable of developing satisfactorily in the rind tissues. Restricted spotting may appear when the rind is fully coloured but the virulent spreading type of lesion only at full maturity when senescence is approaching.

Pearson and Robertson (1954) and Hulme (1954), dealing with apples, consider that the climacteric rise in respiration can be attributed to a stimulus to increased protein synthesis occurring at this time which results in a lowering of the ATP/ADP ratio. This explanation contrasts with that of Millerd, Bonner, and Biale (1953), who considered ripening in the avocado a degenerative process in which respiration is disassociated from phosphorylation and energy transfer, and the ATP supply thereby reduced to a level inadequate to maintain the metabolism characteristic of unripe fruit. Later work by Rowan, Robertson, and Pratt (1957) and others supports the first hypothesis by showing that phosphorylation continues during the climacteric.

An increase in respiratory activity is also a characteristic response of a plant to the attack of a parasite. In reviewing this phenomenon, Uritani and Akazawa (1959) state that respiratory increase is not an attribute of a few host-parasite combinations but a general responsive reaction of plant tissue attacked by pathogenic micro-organisms, including fungi, bacteria and viruses. Furthermore, simple chemical treatment and mechanical stimulus is also able to induce an increase in respiratory rate.

Allen (1953) cites a number of cases in which an increase in respiration has been demonstrated in storage tissue affected by parasitic fungi and deduces that most of the increases can be attributed to the host cells and that the increase is elicted by diffusible substances originating from the parasite. He considered there was evidence that the respiratory increase results from an uncoupling of respiration from the energy-supplying phosphorylations and their regulatory system such as occurs from the action of 2,4-dinitrophenol and ethylene.

In the case of parasitism characterized by an increase in synthetic processes the uncoupling explanation is not wholly acceptable, and in later reviews Allen (1954, 1959*b*) considered that increased synthetic activity itself, by maintaining a rapid turnover of co-factors, stimulated the high respiratory rate. A full explanation of the respiratory increase associated with parasitism based on satisfactory evidence is still awaited (Millerd and Scott 1962).

Shaw and Samborski (1956) demonstrated by the use of radioactive solutions that sugars, amino acids and organic acids were amongst the various products accumulating at the site of infection of the obligate parasites *Puccinia* and *Erysiphe* and considered that this accumulation is directly linked with the enhanced respiratory activity at these sites. In these experiments the few facultative parasites

investigated showed relatively minor accumulations, but Uritani and Akazawa in the review abovementioned cite several instances where the tissue of potato and sweet potato infected by this type of parasite has shown increased synthesis of protein and activation of enzymes.

Kiraly and Farkas (1959) regard the increased respiratory activity occurring in some host-parasite relations as a defence reaction, since abnormal fungitoxic compounds may be produced as a result of the increase in oxidative processes. This is well illustrated by the work with barley mildew carried out by Scott, Millerd, and White (1957). Such an interpretation does not hold in the case of the ripe fruit rots discussed here.

It will be seen from the above brief discussion that the physiological changes occurring in fruit during the ripening process could have features similar to those produced in plant tissue invaded by a fungus and presumably acceptable to the activities of the parasite. Increased respiration is a common characteristic and in general similar explanations for this increase have been forthcoming. The metabolism of the plant cell in storage organs is normally regulated by the phosphorylation system to a conservative maintenance programme. An alteration of the stabilizing mechanism coupled with increased respiratory activity could enable energy and food reserves of the host to be made available to the parasite. Furthermore, the proceeds of the increased metabolic activity may be of a highly organized nitrogenous nature.

On this basis it could be argued that the changes during fruit ripening which commence with the climacteric favour the development of a parasite by providing a metabolic state of the host which it might conceivably duplicate by its own efforts if only provided with an adequate invasion potential. An important consideration is the possibility of the fungus making use of energy derived from the increased respiration which would normally be channelled to plant activities. For a parasite to find the metabolism of its host already travelling along the desired pathway must effect a considerable economy in its own meagre resources. This is an important factor which must be taken into account when attempting to explain the appearance of so many fruit rots during the ripening stage. Other factors such as nutritional changes and alteration in cell-wall characteristics cannot be neglected, but these are not of such overall application.

(b) Effect of Various Physiological Reagents on *Gloeosporium musarum* Development

In Table 19 are grouped a number of compounds which have no obvious connection with nutrition, tissue breakdown or direct toxic action but which may have a distinct physiological effect on the host tissue in some cases associated with respiratory change.

It is well recognized that *2,4-dinitrophenol* at low concentrations (*ca* 10^{-5}M) is capable of uncoupling respiration from the energy-rich phosphorylations and the synthetic processes dependent on these. Interference with the normal regulatory mechanism in this way leads to an increase in the respiratory rate.

TABLE 19

EFFECT OF VARIOUS SOLUTIONS APPLIED TO INOCULATED GREEN BANANAS ON THE DEVELOPMENT OF *Gloeosporium musarum*

Material and Concentration	Rating	No. of Trials	Comments
2,4-dinitrophenol 0·02%	+(1); ++(4)	5	Slightly phytotoxic; highly fungitoxic
2,4-dinitrophenol 0·002% ..	+	6	Not phytotoxic; slightly fungitoxic
2,4-dinitrophenol 0·0002% ..	0	2	Nil fungitoxicity
2,4-dinitrophenol 0·02%, mango ..	+ (2); ++(1)	3	Inoculum *C. gloeosporioides* ex papaw
2,4-dinitrophenol 0·02%, papaw	+(2); ++(1)	3	Inoculum *C. gloeosporioides* ex papaw
2,4-dichlorophenoxyacetic acid 0·02%	+++	1	Slight macerating ability; Moderately phytotoxic
2,4-dichlorophenoxyacetic acid 0·01%	++	1	Nil macerating ability; nil phytotoxicity
2,4-dichlorophenoxyacetic acid 0·01% (immersion)	+++	1	
2,4-dichlorophenoxyacetic acid 0·001%	++	1	
Maleic hydrazide 1·0%	++	2	
Maleic hydrazide 0·5%	++	2	Nil fungitoxicity
Maleic hydrazide 0·05%	0	2	
Potassium cyanide 1·0% (before)	++; +++	2	
Potassium cyanide 1·0%	0; +	2	Highly fungitoxic
Potassium cyanide 0·25%; 0·1%	0	2; 1	Moderately fungitoxic
Sodium benzoate 0·5%	++	2	Highly fungitoxic
Sodium benzoate 0·25%	++	1	Slightly fungitoxic
Sodium benzoate 0·05%	0	2	Nil fungitoxicity
Disodium ethylenediamine tetraacetate 0·1%	0 (1); ++ (2)	3	Nil macerating ability; fungitoxic
Disodium ethylenediamine tetraacetate 0·05%	+ (2); ++ (2)	4	Slightly fungitoxic
Benzimidazole 0·1% (before) ..	0	1	
Benzimidazole 0·1%	—	1	Highly fungitoxic; retarded colouring
Benzimidazole 0·01%	0	2	Nil fungitoxicity
Benzimidazole 0·01% (immersion)	0	1	
Kinetin 0·01%	0/—	2	
Kinetin 0·001%	0/—; 0	2	
White oil 0·6% (v/v)	—(1); 0 (3)	4	
White oil 0·6% (immersion) ..	—; 0	2	
Sterile water plus:—			
Nil wounding	0	3	
Standard pricking	0	many	In the check fruit occasional circles showed development = +
Wounding (various types) ..	0 (2); + (2); ++ (6)	10	
Large area of peel removed ..	+++	5	
1 cm circle of peel removed ..	++	2	

The change in the respiratory pattern brought about by DNP has been likened both to those taking place in fruit at the climacteric (Millerd, Bonner, and Biale 1953; Neal and Hulme 1958) and to the metabolic changes in a host plant associated with parasitic attack (Allen 1953). It would be expected therefore that treatment of fruit with DNP would assist fungal invaders of the ripe fruit rot type. Referring to Table 19 it will be seen that DNP did markedly accelerate the development of *G. musarum* in the banana (Figure 4). Maximum effect was obtained at concentrations higher than that at which DNP is considered to exert its uncoupling effect. The effective strength of the solution as it penetrates the tissue is of course unknown, but it is possible that it was responsible for more degeneration than might be expected from a redirection of respiratory activity alone.

Fig. 4.—Reaction of *Gloeosporium musarum* to certain physiologically active solutions (Table 19). M2, white oil 0·6%; J2, 2,4-dinitrophenol 0·025%; K1, 2,4-D 0·001%; D2, maleic hydrazide 1%.

There have been other examples of DNP lowering the resistance of plants to fungus attack. For example, Gothoskar *et al.* (1955) have broken down resistance of tomato cuttings to *Fusarium* by immersing them in a solution of 10^{-5}M DNP for several days. They suggest that in this case resistance depends on a labile substance depending for its formation on the energy derived from the phosphorylation reactions.

2,4-dichlorophenoxyacetic acid produced the most spectacular acceleration of *Gloeosporium* development of any of the compounds used in the banana circle tests. Evidence for the effect of 2,4-D and other auxins on plants is as yet incomplete. They are generally regarded as exerting a profound effect on plant metabolism and to promote large changes in protein, carbohydrate and enzyme content. These changes have been associated with both increased and decreased resistance to fungal attack, the outcome varying with the particular host-parasite relationship (Davis and Dimond 1952). French and Beevers (1953) have recorded a stimulation of respiration by 2,4-D and other growth regulators when experimenting with corn coleoptiles. This was thought to be due to the stimulation of anabolic reactions requiring high-energy phosphate rather than to an uncoupling action as in the case with DNP.

The action of 2,4-D on banana fruit has been investigated by several workers (Mitchell and Marth 1944; Freiberg 1955; Blake and Stevenson 1959) and it has been well established that the action of this growth regulator is to accelerate ripening. The application of 2,4-D in the circle tests may be regarded therefore as inducing a physiological state similar to that occurring during normal ripening and thus providing conditions resembling those naturally responsible for the breaking of latency. As would be expected, this results in accelerated development of *Gloeosporium.*

Maleic hydrazide has the property of anti-auxin and inhibits cell division and growth, notably of the growing point. As a result of these disturbances there is commonly an accumulation of sucrose, starch and free amino acids. Respiration appears to be retarded or to show only a slight increase.

Samborski and Shaw (1957*a*) investigated the effect on stem-rust susceptibility by adding maleic hydrazide to sand cultures of Khapli and Little Club wheat. A general stunting of growth occurred, but whereas the rust susceptibility of the susceptible Little Club was not altered there was a marked increase in susceptibility of the resistant Khapli. These authors (Samborski and Shaw 1957*b*) investigated the metabolic changes taking place in the treated wheat and made the interesting observation that those induced in Khapli by maleic hydrazide bear a general resemblance to those which naturally occur at the loci of type 4 rust infections on Little Club, namely an increase in dry weight, sugars and soluble amino compounds, the formation of starch and an increase in respiration.

There have been other reports of increased susceptibility following maleic hydrazide treatment. Sitterly and Shay (1960) found that spraying apples on the tree with 100 p.p.m. of the sodium salt of maleic hydrazide retarded the respiratory rate and brought forward the point of climacteric changes. Four inoculated fungi (including *G. cingulata*) were able to commence development at this point one or two weeks before infection showed up in untreated fruit. Hale, Roane, and Huang (1962) report an increase in size and number of leaf spots on susceptible and resistant varieties of maize inoculated with *Helminthosporium carbonum* following treatment with 3, 30, and 300 p.p.m. of maleic hydrazide.

The acceleration of development of *G. musarum* obtained in the banana circle tests is therefore in accordance with results obtained by other workers with different host-parasite combinations. The underlying causes may be somewhat different in the case of maleic hydrazide as compared with DNP and 2,4-D, as the respiratory and energy changes in those cases investigated are less marked.

Potassium cyanide produced marked acceleration when applied at 1·0 per cent. concentration before inoculation. The same concentration applied after inoculation failed to do this, probably because of its highly toxic effect on the fungus. The lower concentrations used showed no reaction. Potassium cyanide in low concentrations inactivates iron- and copper-containing enzymes and has a variable effect on respiration. At a 1·0 per cent. concentration the effect should be a drastic one and akin to serious tissue wounding, which is probably the explanation of the development of *G. musarum*. The failure to produce an effect with lower concentrations is less easily explained but is in line with the action of other polyphenoloxidase inhibitors (Table 8).

Sodium benzoate at the concentrations causing acceleration probably induced profound metabolic disturbances and here again the action may have been partly that of wound stimulus.

The chelating agent *disodium ethylenediamine tetraacetate* gave variable results. In those experiments where acceleration was poor the skin retained its green colour longer than normally in the vicinity of the treated area. Ginzberg (1958) and Letham (1960) have shown that 2 per cent. EDTA at 45° C and pH 10 will cause a separation of plant cells, possibly by chelating the calcium and magnesium in the pectates of the middle lamella. No macerating properties were shown under the conditions employed here and the marked acceleration obtained in some of the experiments is more likely to be due to other physiological effects such as those recorded by Lieberman and Biale (1955) affecting ATP-ase activity.

Person, Samborski, and Forsyth (1957) have shown that *benzimidazole* retards respiration and also chlorophyll and protein breakdown and extends the life of wheat leaves floated in a 30–100 p.p.m. solution. Nitrogen metabolism is stabilized and amino acids are present in smaller amounts than when water alone is used. When benzimidazole is applied to the green banana there is a tendency for the skin adjacent to the treated area to remain greenish while the rest is colouring. It is consistent with a hold-up of the physiological activities associated with maturation and ripening that benzimidazole effects no acceleration in the development of *G. musarum*.

Kinetin, like benzimidazole, delays the approach of senescence and prolongs the life of the treated tissue. As would be expected, no acceleration of lesion development followed its use.

White oil of a high unsulphonated residue as developed for use as an insecticidal spray has come into prominence in recent years as a spray for the control of certain fungus diseases, notably *Mycosphaerella musicola* in the banana leaf. The oil itself is non-toxic to the fungus, so the control obtained must depend on some physiological effect on the host (Pont 1960).

Descriptions of the effect of petroleum oils on plants have been somewhat conflicting owing to the variations obtained with different types of oil and with different concentrations. However, it is generally agreed that the effect of white spraying oil applied at normal concentration is to depress respiration. Photosynthesis is also inhibited. This has been observed with citrus (Knight, Chamberlin, and Samuels 1929; Wedding, Riehl, and Rhoads 1952) and deciduous fruits (Oberle *et al.* 1944).

The simplest explanation for this action of the oil is a mechanical interference with the conducting system and the interchange of gases, although there may be some destruction of chlorophyll and a solubilization effect on the plasma membrane. One result of the retarded metabolism may be seen in the retention of green colour in the skin of treated citrus and banana fruit which otherwise would be yellowing.

In the banana circle tests white oil showed no acceleration and in some cases even a retarding effect on anthracnose development (Figures 4 and 5). This is no doubt due to retarded metabolism and a delay in the ripening process associated with the application of the oil. This state of affairs is the reverse of that described for 2,4-D.

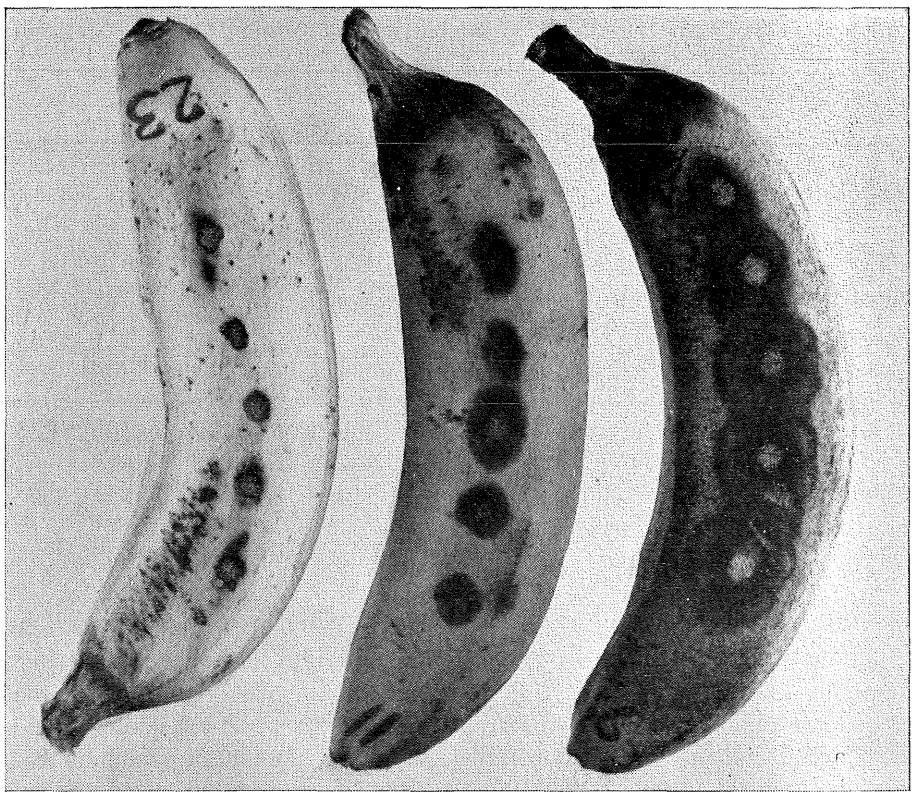

Fig. 5.—Suppression of *Gloeosporium musarum* development by white oil 0·6%. Fruit 23 uninoculated, immersed in water 5 hours. Fruit 11 and 6 spray inoculated, then 11 immersed in white oil, 6 in water. Fruit 11 yellowish green, 6 and 23 yellow when photographed. Note development from both pricked and unwounded surfaces in Fruit 6.

(c) Methyl bromide and the Breaking of Latency

When methyl bromide was introduced as a fumigant to destroy fruit fly in susceptible fruit shipped from Hawaii to the U.S.A. it was found that in the case of the papaw this treatment had the effect of breaking the latency of the *Colletotrichum* infections in the green fruit so that fungal spotting made its appearance earlier and more abundantly than on those not fumigated. So characteristic was this action that Parris and Jones (1941) suggested fumigation with methyl bromide (2 lb/1000 cu. ft. for 3½ hr) as a means of detecting latent infections and used as illustrations *Colletotrichum* spp. on the papaw and *C. lindemuthianum* in bean.

Ethylene dibromide has been similarly implicated. This fumigant has been found to increase stem end rot in citrus (Hopkins *et al.* 1957) and to break the latency of black spot (*Guignardia citricarpa*) infections in Valencia oranges (Trout 1958).

Lindgren and Sinclair (1951) have shown that appreciable quantities of bromine are absorbed and retained by citrus and avocado fruit following methyl bromide fumigation. Methyl bromide being unstable, a portion is broken down releasing bromine, which reacts to form various inorganic and organic compounds. There exists therefore a good basis for expecting physiological changes to take place in the fruit as a result of the fumigation.

Methyl bromide applied to apples in the preclimacteric stage at dosages of ½ lb to 2 lb/1000 cu. ft. for 5 hr increased respiration over the first four days (Southwick 1945). Enzymes containing an SH group, of which there are a number associated with respiration, are inactivated by methyl bromide (Lewis 1948). As a further point, Deuel and Stutz (1958) state that pectin is vigorously attacked by bromine.

The breaking of latency by methyl bromide could be explained therefore by physiological changes brought about by bromine absorbed through the skin of fumigated fruit. The exact factor responsible is a matter of conjecture but it is probably bound up with respiratory changes, which have been shown to be important in other circumstances.

(d) A Physiological Basis for the Effect of Wounds on Latent Infection

It is a well-known fact that wounding and bruising will increase the susceptibility of fruit to the ripe fruit rots and care in handling is one of the few procedures militating against loss from these troubles. Artificial inoculation of the Cavendish banana has shown that when the amount of inoculum is large there is little difference in the number of infections produced when it is applied to wounded or apparently uninjured skin. Using a smaller number of spores,

more akin to conditions in the field, infection is more readily achieved on a wounded surface and the resulting lesion usually develops more rapidly (Simmonds and Mitchell 1940) (Figure 6).

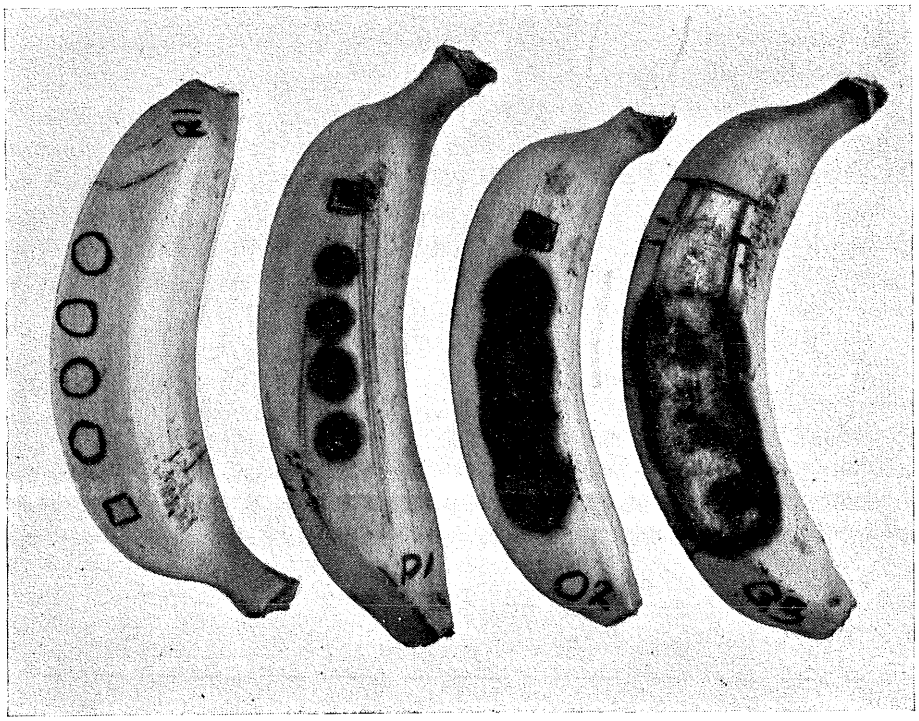

Fig. 6.—Effect of various types of wounds on the development of *Gloeosporium musarum*. R1, unwounded; P1, surface scorched; O2, surface lacerated; Q3, peel removed.

Meredith (1960) has described a restricted type of lesion which may develop in the wounded skin of the green Lacatan banana. He refers to this as "non-latent anthracnose", inferring that infection is direct with no latent phase. As will be shown later, wounded tissue is in a state which would permit the direct invasion described by Meredith, but in the case of fruit already carrying numbers of latent infections at the time of harvest a proportion at least of the "non-latent" lesions in the green fruit could be the result of a stimulus into activity of latent infections already present at the site of the wound.

The assistance offered by an injury to fungal attack is commonly explained on the basis that dead or dying tissue serves as a better substrate for development than does the living cell. This is no doubt correct in many cases but with *Colletotrichum* a somewhat different explanation is possible.

Richards (1896) investigated the effect of wounding on respiration in connection with potato, carrot, bean and pumpkin and the leaves of several plants and recorded that after injury to plant tissue there occurs a marked

increase in respiration varying in intensity and duration with the character of the tissue involved and with the extent of wounding. The increased activity usually reaches a maximum in about two days and then falls gradually to almost normal as the wound heals. Johnstone (1925) points out that some of the increase in respiration following wounding may be due to a mechanical facilitation of the exchange of gases. Pearson and Robertson (1954) obtained increased respiration from sliced apple tissue compared with the intact fruit. Burg (1962) reports that in the case of cut tomatoes and peeled bananas there is a marked increase in carbon dioxide and ethylene production commencing a few hours after wounding and lasting for several days.

Williamson (1950) investigated ethylene production by diseased, injured, and healthy tissue of 12 species of plants. Healthy leaves produce small quantities of ethylene, the amount depending to some extent on the species. Diseased tissue and leaves which have been injured by shredding showed a marked increase in ethylene production. In the former case the increase is thought to be due to the injury to the tissue brought about by the presence of the pathogen. Ethylene is used commercially to accelerate colouring and ripening in fruit and vegetables. It is produced during the natural ripening of many fruits and Burg (1962) considers that accumulated evidence favours the opinion that an increase in ethylene production at the onset of the climacteric may be causally related to the ripening process.

In the case of wounds it is conceivable that the ethylene produced coupled with the increase in respiratory rate would induce a physiological state somewhat akin to that in normal ripening. The yellowing and softening which often takes place adjacent to a wound lends support to this contention. A more specific explanation of the action of wounding in allowing early development of the ripe fruit rots is therefore to be found in the physiological changes brought about by the wound stimulus whereby the normal state of synthesis is replaced by one characterized by an increase in respiration and other maturation effects which are known to provide suitable conditions for *Gloeosporium* development.

VII. DISCUSSION AND CONCLUSION

In dividing up this investigation of the problem of latent infection into the four aspects of nutrition, enzyme potential, toxins, and respiratory activity, the main factors likely to be contributing to the phenomenon should have been covered. Reviewing then the information provided, it is disappointing that no single, well-defined explanation has evolved. Each of the aspects dealt with here, if taken on its own, provides evidence which might be, and sometimes has been, taken as a possible cause. It is only when each is viewed in relation to the others that the subject assumes complexity and no single explanation appears fully adequate. The situation is one which might well be covered by the balance hypothesis propounded by Lewis (1953), in that the outcome of latent infection is probably determined by the complex interplay between the metabolites of host and parasite involving both useful and inhibitory substances.

Commencing with nutrition, it is fairly certain that *G. musarum* could develop satisfactorily on the starch and other simple nutrients in the green banana tissue if its initial invasion potential was sufficient to enable it to avail itself of this source. This low invasion potential can be augmented by applying to the inoculated surface nitrogenous compounds which provide the parasite with a highly organized food base without the necessity of expending its own meagre resources on its synthesis. However, as already pointed out there is no evidence of a sufficiently marked difference in the nitrogenous compounds of the green and the ripe fruit to account for the sharp demarcation in the parasitic activity of *G. musarum* in the two cases.

The question of enzyme potential is one which it is difficult to separate from that of nutrition. At first sight the acceleration produced by Pectinol and the solution from below some fungal cultures would suggest that the failure of *G. musarum* to develop past the latent stage was due to a lack of the macerating enzymes required to effect penetration of the green tissue. This was supported by evidence that *Colletotrichum* species are relatively poor producers of macerating enzymes and pectinesterase *in vitro*. By employing these same solutions after inactivating the enzymes by heat it became apparent that, provided the substrate was adequate, *G. musarum* was capable of providing an effective enzyme supply without assistance from an outside source. The fundamental factor is a nutritional one.

Assuming that the initial penetration of the fungus is by way of the middle lamella and primary cell wall, the solubilization of the pectic constituents of these as ripening proceeds may be a factor in the final breaking of latency.

The question of the existence of an active anti-fungal toxin is one of the most intriguing and at the same time one of the most difficult propositions to prove. Although toxicity is not manifest in cold extracts of the green banana skin, it appears from the evidence of hot extracts that the basic requirements for the production of a toxic substance do exist. In the banana these seem to consist of a substrate not always available and a heat-sensitive enzyme. If a toxin is to be implicated as a cause of latency it is necessary to assume either that it is too closely bound up in the tissues of the host to be available in cool extracts or that it is not present in an active state in the green fruit and is only brought into operation as the result of host-parasite interaction.

Some circumstantial evidence lends support to a toxin theory: for example, the disappearance of extractable toxin as the fruit ripens; the existence of the toxin or its precursor in the outer layers of the skin; the correlation in some instances of toxic activity with anthracnose susceptibility; and the existence of toxic substances in somewhat similar circumstances in other fruits subject to latent infections.

The existence of an anti-fungal toxin in green but not ripe fruit would fit in well with the knowledge of the behaviour of latent infections. However, until further investigation has provided more information on the chemical nature

of the toxic substance and its location in the tissue of the plant, it would be prudent to regard it as one of the several possible environmental conditions contributing to latency rather than playing a major role.

The respiratory and energy changes taking place during the ripening of fruit are more fundamental and less subject to simple interpretations than the aspects already dealt with. On the other hand, their fundamental nature is an argument in favour of their serious consideration in connection with a subject having as wide an application as does latent infection. There is reason for believing that the metabolic activity characteristic of the climacteric and post-climacteric phases of fruit development is not far removed from that arising as a result of parasitic invasion and presumably satisfactory to the needs of the invader. This environment enables the metabolic activities of the parasite to proceed at a pace far in advance of that possible in normal green tissue. The accelerated development of *G. musarum* produced by the application of such substances as DNP and 2,4-D known to effect respiratory activity and general metabolism lends support to this hypothesis. Wounds which are conspicuous for the stimulation they give to *Gloeosporium* development probably function by providing metabolic conditions similar to those occurring during normal ripening.

Associated with the respiratory change and probably dependent on this are such factors as nutritional changes, solubilization of cell-wall constituents and the denaturing of toxic substances, all of which may play a part even though a subsidiary one.

The situation may therefore be summarized as follows:—

Within the genus *Colletotrichum* there exists a group of facultative parasites possessing a relatively low initial invasion potential especially with respect to their ability to secrete macerating enzymes in the absence of a suitably organized substrate. With the assistance of the appressorium, an organ particularly well developed in this genus, the individual is able to attach itself to the epidermis of its host and proceed to the formation of an infection thread which penetrates the cuticle and comes to lie on or between the cellulose layers of the outer cell wall. The subcuticular hypha forming the latent stage is a small knot of mycelium containing part of the resources of the original spore but nothing more. In this it is less favoured than those fungi which build up a more imposing hyphal structure before commencing the act of penetration.

If on green fruit the parasite is now faced with an immediate environment lending little assistance to further development, the host metabolism is in a conservative state of synthetic equilibrium providing no readily available energy source. Supplies of the more highly organized nutrients in the immediate vicinity are inadequate or inaccessible and there is the additional possibility that the fungus is confronted with substances inhibitory to its own metabolic activities. Under these circumstances no further development of the parasite takes place and no doubt many of the original latent infections never regain activity.

With the onset of ripening the situation undergoes a marked change in favour of the invader. Energy resources become available due to alterations in the mechanisms regulating host metabolism. Additional nutrients may be elaborated or moved to the site of invasion, pectic materials become more accessible to the action of enzymes, and the risk of encountering toxic barriers is eliminated. With this outside assistance the parasite is able to make full use of its own slender resources and proceed to further development.

In conclusion, one factor often left out of consideration might be mentioned. The capacity to form latent infections which are both expedient and remunerative undoubtedly has had a phylogenetic origin in the genus *Colletotrichum* comparable with that of the appressorium and reproductive organs. This innate faculty for forming the subcuticular hyphae must contribute in no small degree to the uniform behaviour exhibited by individuals in this phase if their development. Such behaviour is not necessarily entirely determined by the environment.

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