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The Fungal Contamination of Kentish Strawberry Fruits in 1955

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Fungal rotting of strawberries during transport and marketing is a major factor limiting the area from which manufacturers of jams and preserves can draw supplies of fruit. Furthermore, the presence of mold mycelium in or on the fruit is of special importance to manufacturers in the United Kingdom who export to certain overseas markets, notably the U. S. A., where the acceptability of their products may be partially governed by the mold content. This is generally determined by the Howard mold count technique (*Official Methods of Analysis of the Association of Official Agricultural Chemists,* 1950), which has been generally accepted as providing an index of the extent to which fruit used for manufacture has been subject to deterioration caused by fungi. Howard (1917) correlated mold counts with the extent of rotting in tomatoes, that is, decomposition associated with the activities of living microorganisms. Needham and Fellers (1925) extended the use of the Howard technique to soft fruit products but used the term "moldy berries" apparently as a synonym for fruit rotted by fungi. The word mold is a popular term with no exact definition, but is often taken to describe "a microfungus having a well marked mycelium or spore mass, especially an economically important saprophyte" (Ainsworth and Bisby, 1950). It appears possible, therefore, that the emphasis placed on the undesirability of the presence of fungal hyphae as indicating rotting may have become placed on the undesirability of hyphae as such.

Beneke *et al.* (1954) investigated the relation of the fungus flora to pectolytic breakdown in Michigan strawberry fruits, and concluded that the amount of mold determined by the mold count may not necessarily be related to the extent of breakdown in a food product. It may be suggested that a high mold count may indicate poor handling conditions in so far as such conditions may favor the development of otherwise harmless superficial fungi, but it is also possible that a mold count may be increased by contamination of the fruit in the field by fungi such as the powdery mildews which may not cause rotting and may often be considered not to affect quality. Furthermore, the mold count gives no indication of the type of softening and breakdown which sometimes occurs in strawberries held in solutions of sulphur dioxide, because although enzymes liberated by microorganisms at an earlier stage may be responsible (Pandhi, 1953), the extent of breakdown in susceptible fruits is partially dependent on the length of time that they are held in the preservative solution.

The present investigation was undertaken to determine the organisms responsible for the rotting of strawberries harvested in mid-Kent during 1955, together with the significance, relative to the Howard mold count, of the mycelium of fungi not associated with rotting.

It would normally be preferable that the control of mold growth on strawberries during transit or storage should be carried out by modifying environmental conditions such as temperature. In practice, however, it may not always be possible to obtain adequate control by such means, and a search was made for a suitable method of chemical control. The application of chemicals to foodstuffs is strictly limited in the U. S. A. by the Food and Drug Acts, and in the United Kingdom by the Public Health (Preservatives in Food) Regulations, and the problem was therefore approached by

[1] This work was carried out during the tenure of a Treasury Senior Research Fellowship as part of the program of the Food Investigation Organization of the Department of Scientific and Industrial Research.

investigating the possible use of rapidly dispersed volatile fungicidal agents.

Materials and Methods

Beneke *et al.* (1954) assessed the relative prevalence of species of fungi by incubating standard plugs of fruit tissue placed on an agar medium and by counting and identifying the resultant colonies. Obligate parasites are not detected by this method, and fungi present only as spores are given equal prominence with those present as mycelium. As the presence of mycelium is the sole basis of the Howard technique, Beneke's methods cannot be directly equated with those obtained by a Howard mold count. In the present work, the Howard technique was followed as closely as possible with adaptations to permit the specific identification of mycelial fragments and spores.

Samples of strawberry fruit were obtained from four sources in mid-Kent on two occasions, early (var. Royal Sovereign) and late (var. Auchincruive Climax) in the season. The fruit was protected by a sheet of polythene film and carried directly to the laboratory for testing as soon as possible after it had been picked. For each test a sample of at least 4 lbs of fruit was obtained by random selection of individual fruits from a large bulk. Two lbs were examined immediately after picking, a further 2 lbs being held at 20 C for 48 hours before examination. To make the examination, 1 lb of fruit was hulled and macerated in a sterile Waring blendor for 20 minutes. The product was then incorporated, by the methods normally employed in the preparation of dilution plates, in sterilized 2 per cent malt agar containing a minimum quantity of agar. Normally 10 per cent by volume of the fruit was incorporated in the agar, but greater dilutions were used if fungi were prevalent. Aliquots were then poured to form a thin layer in sterilized Petri dishes. The prepared plates were incubated at 20 C and examined under the microscope at intervals. When viable mycelial fragments or spores were observed to start growth they were cut out individually by a microscope nosepiece attachment, using aseptic techniques, and transferred to 2 per cent malt agar slopes. The resultant cultures were retained until specific identification was possible. The origin of each culture, that is, from mycelium or from spore, was carefully determined before isolation and this was found to necessitate frequent examination of the plates. Approximately 70 cultures were made from each sample, but, with experience, the identity of common species could be determined without isolation, and this was carried out only in case of doubt.

One pound of fresh fruit from each sample was examined under a low power binocular microscope and any sporulating fungi were noted. The fruits in a further pound were plated out individually under aseptic conditions on moist, sterile filter paper in large Petri dishes. This fruit was then incubated at 20 C and examined at intervals for rotting and the presence of fungi.

All of the species of fungi isolated during these tests were investigated with a view to determining their ability to cause rotting of inoculated fruits. Sound, ripe, strawberry fruits were surface sterilized by a 5-second dip in an alcoholic 0.1 per cent solution of mercuric chloride, followed by a 15-minute wash in each of two changes of sterile, distilled water. The fruits were then transferred under aseptic conditions to sterile Petri dishes. The pathogenicity of each fungus was then tested by inoculating 10 of the prepared fruits with a fragment of a pure culture, five fruits being undamaged, and five being wounded by pricking them with a sterile needle at the site of inoculation. Ten intact and 10 wounded fruits were left as uninoculated controls. All of the fruits were then incubated for 2 weeks at 20 C in a damp chamber.

In the examination of fungicidal treatments, two 1-lb samples of fresh fruit, drawn by random selection of individual fruits, were used for testing each treatment in each experiment. Two liters of each solution to be tested for fungicidal activity were placed in polythene buckets, and the fruit, contained in punnets, was immersed and gently agitated for approximately five seconds. The fruit was then withdrawn, surplus fluid drained off, covered by a sheet of polythene film, and incubated at 20 C for 48 hours. In each experiment, 2 lbs of fruit were left untreated and 2 lbs were dipped in sterile water to serve as controls. After the period of incubation, 1 lb of the fruit representing each treatment was submitted to a tasting panel and the second 1-lb sample was submitted to a Howard mold count after counting and removing any severely rotted fruits.

The chemicals investigated included acetaldehyde, ethyl alcohol, peracetic acid, and a saturated solution of citric acid in 20-vol hydrogen peroxide. All chemicals were diluted to the required strengths with distilled water.

Results

Although the absolute quantity of mycelium found to contaminate the various samples was found to vary, the species of fungi and their relative preponderance was similar in all of the samples examined.

With freshly picked fruit, from 50 to 95 per cent of the mycelial fragments were not viable in an agar medium. When the fruit had been stored at 20 C for 48 hours, although the proportion of sterile to fertile hyphae decreased, the number of sterile fragments per unit volume remained approximately constant, and it was concluded that the sterile mycelium was that of an obligate parasite. *Sphaerotheca humuli* (DC.) burr was observed on the surface of much of the fruit examined and, as the sterile mycelium found during the examination of the agar plates was similar morphologi-

cally to that of a powdery mildew, it appeared probable that this species might contribute substantially to a Howard mold count. A sample of fruit bearing sufficient *S. humuli* to be just visible to the naked eye, but with no other blemish, was carefully selected and submitted to a Howard mold count, and a similar sample was examined by the plate technique. The mold count averaged 34 per cent of positive fields, while 94 per cent of the hyphal fragments in the parallel plate test proved sterile. A mold count of 15 per cent was obtained from a similar sample bearing little visible mildew.

Throughout all the tests 60 to 80 per cent of the viable mycelial fragments produced colonies of *Mucor piriformis* Fischer, and 96 to 100 per cent of the incubated whole fruits were found to be contaminated by this species, which was the most important cause of rotting in the samples examined.

All but approximately 1 per cent of the remaining viable hyphal fragments produced colonies of *Botrytis cinerea* Pers. ex Fr., which was found to contaminate from 45 to 70 per cent of the incubated whole fruits. Because of the rapidity with which *Mucor* attacked the fruit, *Botrytis* was rarely the primary cause of rotting, but developed slowly by intermingling with the established *Mucor*.

The remaining viable hyphal fragments, comprising from 0.5 to 2 per cent of the whole, were composed of *Cladosporium herbarum* Link ex Fr., *Penicillium* spp., and *Trichoderma viride* Pers. ex Fr. On the whole fruits, the development of these species was practically entirely restricted to the senescent styles adhering to the fruits, sporulation taking place on the stigmatic surfaces. During incubation, a few hyphae were observed to develop from these small colonies over the adjacent surface of the fruit, but these had little influence on the mold count.

The conidia of *Cladosporium*, *Penicillium*, and *Trichoderma* were very common in preparations of all of the samples examined, but conidia of *Botrytis*, while frequently found in preparations made from fresh fruit, were less frequent in preparations from fruit kept at 20 C for 48 hours, probably due to their having germinated. Spores of *M. piriformis* were occasionally found, and those of *Alternaria* sp., *Epicoccum* sp., and *Pullularia pullulans* (De Bary and Low) Berkhout, were each isolated on one occasion.

A yeast, *Kloeckera apiculata* (Reess emend Klöcker) Janke, was found to develop abundantly on injured fruits and was by far the most common single organism found in the plate tests.

When the pathogenicity of the various species was investigated, severe rotting developed only in those fruits inoculated with *B. cinerea*, *M. piriformis* and *Kloeckera apiculata*, the first two species attacking both wounded and unwounded fruits while the yeast attacked the wounded fruit only. The rot caused by *Botrytis* is too well known to warrant description, but those caused by *M. piriformis* and *K. apiculata* are of interest. Both of the latter, in the early stages of attack, caused the surface tissues to soften and become paler in color. *M. piriformis* broke down the fruit to a soft mush within four days at 20 C, but aerial hyphae and sporangiophores did not appear until breakdown was nearly complete. The rot caused by *K. apiculata* was similar but required 10 to 14 days to reach completion. A cream-colored mass of yeast frequently developed in the inoculation wound (figure 1), which enlarged to form a crater. All of the uninoculated fruits, with one exception, remained sound and healthy throughout the incubation period.

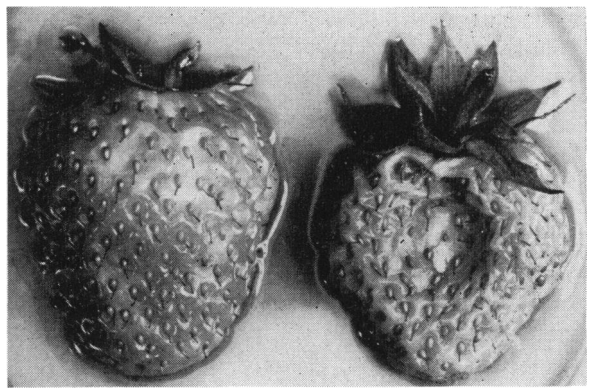

FIG. 1. Breakdown of surface sterilized strawberry fruit, following wound inoculation with *Kloeckera apiculata*. Uninoculated fruit on the left, inoculated fruit on the right, after 14 days incubation at 20 C.

Apart from the eventual development of sporangiophores on the fruit attacked by *Mucor*, the only superficial difference between the rots caused by this species and by *Kloeckera apiculata* lay in the occurrence of leakage from the infected tissues, abundant fluid being liberated from fruits infected with *Kloeckera*, but little escaping from those infected with *Mucor*. Substantially more leakage occurred from fruits naturally infected with *Mucor* but it was found that yeasts were almost invariably also present. In the samples examined, yeasts were rarely a major cause of rotting because of the more rapid development of other rot-producing organisms.

After several small preliminary experiments in which it was found that treatment with peracetic acid appeared to afford a promising method of controlling the growth of fungi on strawberry fruits, two large experiments were carried out using fruit from two different sources. The results of these experiments are given in table 1.

It will be seen that, again, promising results were obtained with peracetic acid at a concentration of 0.5 per cent of the stable 40 per cent commercial product. None of the other substances gave satisfactory control.

No taint or unusual odor could be detected from the

TABLE 1. *The control of molds by dipping strawberry fruits in various chemicals*

Treatment	Experiment I		Experiment II	
	Percentage of severely rotten fruit	Howard mold count	Percentage of severely rotten fruit	Howard mold count
		%		%
Fruit freshly picked	0	15	0	12
Fruit after incubation for 48 hours at 20 C.				
Untreated	17	36	27	32
Dipped in water	20	47	34	24
Dipped in 98 per cent alcohol	—	—	22	17
Dipped in 20 per cent alcohol	—	—	25	23
Dipped in citric acid + H_2O_2 1 per cent	25	24	—	—
Dipped in citric acid + H_2O_2 0.5 per cent	40	43	—	—
Dipped in 1 per cent acetaldehyde	*	*	—	—
Dipped in 0.25 per cent acetaldehyde	—	—	12	39
Dipped in 0.1 per cent acetaldehyde	12	27	—	—
Dipped in 1 per cent peracetic acid	*	*	—	—
Dipped in 0.5 per cent peracetic acid	10	17	7	14
Dipped in 0.25 per cent peracetic acid	—	—	22	8

* Fruit damaged by treatment.

Severely rotten fruit was removed before carrying out the Howard mold counts.

fruit treated with 0.5 per cent peracetic acid after the lapse of 24 hours, although a slight bleaching had occurred in any open wounds on the fruit. After 48 hours' incubation at 20 C, a sample of fruit which had been treated with 0.5 per cent peracetic acid was tested for the presence of peracetic acid or peroxide. Twenty ml of water was repeatedly poured over the surface of ½ lb of the fruit contained in a funnel and no peracetic acid or peroxide could be detected in the washings when analyzed by a method capable of detecting 1 ppm of peroxide[2].

DISCUSSION

Although *Mucor piriformis* was found to be the most prevalent rot-producing fungus in the fruit examined from the 1955 crop, previous experience suggests that this is unusual and that *Botrytis* is usually the predominant pathogen. The reason for the predominance of *Mucor* in 1955 is unknown at present, although the unusually dry weather experienced throughout the season may have been a contributory factor.

The significance of the present results lies in the demonstration that although many small colonies of *Cladosporium*, *Penicillium*, or *Trichoderma* were present on practically every strawberry fruit, theses species had but an insignificant influence on the Howard mold count. In contrast, the possibility of obtaining high mold counts from sound fruit infected with the powdery mildew *Sphaerotheca humuli*, which does not cause a rot, suggests a possible source of error in the Howard mold count technique when used as an index of fruit deterioration. So long as the technique remains in use as a standard, however, it appears that mildewed fruit, even when free from rots, should be used with caution by the jam manufacturer. Where fruit has to be transported or held in storage before manufacture, visibly mildewed fruit should be avoided as the development of even a small amount of rotting might raise the mold count to unacceptably high levels.

Peracetic acid is an aliphatic peracid (CH_3COOOH) which readily breaks down to acetic acid and water while liberating oxygen. Any residual acetic acid left on treated fruit, being volatile, could be expected to disperse during processing. In fact, no acetic acid was detectable by odor or taste on fruit treated with peracetic acid and subsequently held for 24 hours in a 20 C store. Greenspan and MacKellar (1951) obtained good control of molds growing on harvested tomatoes by the application of peracetic acid and extended the method to the prevention of mold growth on grapes. In view of the encouraging results obtained during the present work and as acetic acid is nontoxic, and no residual peracetic acid was detectable after short periods of storage, this substance warrants consideration as a possible fungistatic agent for use during the transport or short period holding of strawberry fruits.

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The writer wishes to thank Mr. C. L. Hinton for an arrangement whereby the majority of the Howard mold counts were carried out by the staff of the British Food Manufacturing Industries Research Association, Leatherhead.

SUMMARY

An investigation was carried out to determine the identity of the fungal hyphae contaminating strawberry fruits harvested in mid-Kent during 1955. In freshly picked fruit the most common hyphae were generally those of *Sphaerotheca humuli* (DC.) Burr which, together with *Botrytis cinerea* Pers. ex Fr. and *Mucor piriformis* Fischer, contributed 98 to 99.5 per cent of the total hyphae both in fresh and stored fruit. *M. piriformis* was, in this season, the most important cause of rotting during storage, with *B. cinerea* contributing in a secondary capacity. *Cladosporium herbarum* Link ex Fr., *Penicillium* spp., and *Trichoderma viride* Pers. ex Fr. were almost ubiquitous on the senescent styles and stigmas adhering to the fruit, but

[2] This method was based on the reaction of hydrogen peroxide with iodide in an acid medium by the reaction $2\ H\ A\bar{c} + H_2O_2 + KI \rightarrow I_2 + 2\ KA\bar{c} + 2\ H_2O$, the liberated iodine being estimated by the coloration of starch.

the proportion of hyphae attributable to these species was insignificant. The most common organism in plate counts was the yeast *Kloeckera apiculata* (Reess emend Klöcker) Janke, which caused slow breakdown when inoculated into wounded, surface sterilized, fruits. The significance of figures obtained by the Howard mold count technique is discussed in the light of these results.

A small scale investigation of substances suitable for application to harvested strawberries, showed peracetic acid to be a promising agent for the control of mold development during short periods of storage.

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The Influence of Sealing Pressure on Survival of *Serratia marcescens* and *Micrococcus pyogenes* var. *aureus* Desiccated from the Frozen State[1]

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Until Naylor and Smith (1946) demonstrated 100 per cent livability of *Serratia marcescens* desiccated from the frozen state, it was generally considered that the per cent livability of lyophilized cultures was low, below 10 per cent. These investigators dried concentrated suspensions of *S. marcescens* in a specified medium at pressures of approximately 2 to 5 microns of mercury. Hutton *et al.* (1951) investigated the effect of the temperature of the subliming ice, the rate of sublimation and the moisture content of the dried product on the per cent viability of *Brucella abortus* strain 19.

The present study was undertaken to learn the effect of sealing pressure on the per cent livability of *S. marcescens* and *Micrococcus pyogenes* var. *aureus* desiccated from the frozen state, using ordinary laboratory equipment and storing for short periods of time. *S. marcescens* was chosen due to its relative susceptibility to the drying process and *M. pyogenes* because of its relative resistance to drying.

[1] Journal Article No. 1835 from the Michigan Agricultural Experiment Station, East Lansing, Michigan.

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MATERIALS AND METHODS

Lyophilization of *S. marcescens* was carried out following the general procedure outlined by Naylor and Smith (1946). The cells were grown in a liquid medium composed of 1.0 per cent Difco tryptone, 0.5 per cent Difco yeast extract, 0.1 per cent glucose, 0.2 per cent disodium phosphate and 0.1 per cent sodium dihydrogen phosphate at a pH of 6.8 to 7.0. This medium was dispensed in 250-ml amounts in 500-ml Erlenmeyer flasks. Stock cultures were maintained on agar slants and incubated at room temperature to obtain good pigment production. Two loopfuls of growth from 24-hour slants were transferred to the liquid medium. This medium was aerated by continuous agitation employing a Burrell Shaker at a frequency of 170 cycles per minute and an amplitude of 16 cm. After 18 to 24 hours of continuous shaking at room temperature, the cells were concentrated by centrifugation, at an RCF of approximately 1,400 $\times$ g, for one hour. The supernatant was decanted and approximately 3 ml of diluent, composed of 0.5 per cent ascorbic acid, 0.5 per cent thiourea, 0.5 per cent ammonium chloride, and 2.0 per cent dextrin, were added to the sediment. The