THE INCIDENCE AND ENZYMATIC ACTIVITY OF MOLDS FOUND IN INDIANA AND OHIO TOMATOES

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This is to certify that the

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By

Lawrence Sinclair White

AN ABSTRACT

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This study was undertaken to determine the genera and species of fungi present in tomatoes and the ability of these molds to produce enzymatic and other changes in tomato fruits. It was thought that the pectolytic enzyme polygalacturonase (PG) might be of more significance than generally has been realized. Also of importance was the relation of the amount of visible root and mold growths to mold counts. Mold counts have been used as a criterion of the quality of tomato products since 1911.

Tomatoes showing mold growths were collected from a widespread growing area in the states of Indiana and Ohio. The genera and species of molds present were correlated with the gross deterioration evident in the tomatoes. The behavior of molds in producing flavor, odor and pH changes was determined. Subsequently sound whole tomatoes were inoculated with the molds and the symptoms were described.

Howard mold counts were made on comminuted tomatoes which showed various fungus genera and known percentages of visible rot. Also the influence of trimming operations on mold counts was studied.

The amount of PG and the enzyme cellulase (Cx) produced by the molds in tomato extracts was determined employing the "cup-plate" method of Reid (1950) and Dingle, Reid and Solomon (1953).

Under field and experimental laboratory conditions the genera and species of molds which were present were of prime importance in determining the presence or absence of rot in tomatoes. Two of the most widely distributed molds in tomatoes, Alternaria solani and Colletotrichum phomoides often produced only minor lesions. By contrast, Oospora, Rhizopus, Fusarium and Mucor sp. which generally do not appear as frequently under field conditions unless the humidity and temperature are high, were found to be the most active molds in producing rot. Tomatoes inoculated with Rhizopus sp. showed the development of cracks.

The genera and species of molds present influenced the pH and the flavor of juice extracted from the field tomatoes and similar changes were observed in inoculated tomato juice samples. While off-flavors usually were detected, some molds produced pleasant flavors and one strain of Penicillium sp. produced a flavor in tomato juice which was preferred by tasters to that of uninoculated juice.

In most instances juice made from tomatoes which contained molds had high pH values and the examination of trims and culls showed appreciably higher pH values than sound whole tomatoes. The increase in the pH of tomatoes which contain molds was suggested as a factor which might contribute to the development of "flat sour" organisms and also subsequent spoilage of tomato juice.

Juice made from tomatoes which had been trimmed invariably showed higher mold counts than juice made from tomatoes which did not require trimming.

High mold counts were found when very little rot was present, e.g., in one instance, as little as 0.1 percent visible rot by weight gave a Howard mold count of 50. While Eisenberg (1952b) made no mention of it, his data showed the same thing in several instances. These data are in contrast to the results of Howard and Stephenson (1917) which indicated mold counts in excess of 50 percent positive fields were present only when 5.5 percent or more visible rot by weight was present. Conversely, our work showed that a high percentage of visible rot may give low mold counts which was also previously shown (Eisenberg, 1952a; Smith, 1952).

The work reported here further demonstrated that there was not necessarily any correlation between the amount of visible rot and the Howard count since it is the type of mold which is the determining factor on the amount of visible rot present as well as having an important bearing on the mold count itself. In short, the type of mold which the Howard mold count does not and cannot determine is a more important consideration than the mere presence of infinitesimal mold fragments which may or may not be important from the standpoint of rot.

The greatest PG activity was found in tomato tissue and in tomato juice samples which contained the most active rot-producing molds. In contrast to cellulase (Cx), the concentrations of this enzyme closely paralleled the activity of the molds which produced the most rapid deterioration of tomato tissue.

A considerable variation occurred in different strains of molds of the same species with respect to PG activity and amount of tissue breakdown in the tomato fruit. An outstanding example of this was found in Colletotrichum phomoides. This was a definite indication that the Howard mold count did not correlate with the amount of rot produced by some of the molds. In many incidences there was less rot produced by the same amount of growth of hyphae in some strains of the mold as shown by PG activity.

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INTRODUCTION

It is well known that some molds have caused extensive damage to foods. It is not so well known that other molds have imparted very desirable characteristics to some foods and have resulted in the attainment of qualities which otherwise would have been impossible in these foods. The familiar Roquefort, Camembert and Blue cheeses are only a few examples of food products created by the activities of different genera, species and varieties of molds. Molds also have fermented foods to yield beverages, some of which have been consumed extensively such as Sake, the national drink of Japan.

In addition molds have been used in the manufacture of gluconic acid, citric acid, fumaric and gallic acids which have been employed in food products. By-products of mold metabolism have been used on a large scale in the clarification and stabilization of fruit juices, jams and jellies.

One of the characteristics of molds is that usually they grow more slowly than bacteria and yeasts. Some workers have used the presence of molds in processed food products as an indication of the condition of the raw produce and the efficiency of trimming and sorting procedures. This criterion of quality was applied to tomatoes and tomato products over 40 years ago and still is employed by government agencies.

In recent years technologists have made considerable progress in developing improved tests for measuring food quality. Many tests formerly employed have been discarded as a better understanding has been gained of the basic principles of food production and manufacture.

This work was undertaken to study the types of molds found in tomatoes, to determine the ability of these molds to cause enzymatic and other changes in tomato fruit and to relate, if possible, the findings to currently accepted interpretations of the Howard mold counts.

The tomatoes were collected principally in the State of Indiana during the 1953 canning season. In terms of dollar value, Indiana is second only to California in the production of processed tomato products in the United States. Processing of tomato products is a major industry as is reflected in the value of the tomato pack which rose steadily from 19 million dollars in 1935 to 134 million dollars in 1951.

PART I

STRUCTURAL CHANGES ASSOCIATED WITH THE PRESENCE OF MOLDS IN TOMATO FRUITS

Introduction

The purpose of this investigation was to examine the types of molds which are commonly found in tomato fruits and to study what structural changes may occur in the tomatoes when mold growth is present. The presence of mold necessitates extensive sorting and trimming in tomato processing factories as all mold filaments, regardless of type, are included in the Howard mold count. This has been used to condemn tomato products as unfit for human consumption.

Review of Literature

Most publications concerning molds common to tomatoes have been concerned with the individual genera and species of molds which have been known to cause disease or decomposition of tomatoes. Some of these publications have been written by plant pathologists or workers who have summarized data for laboratory personnel engaged in mold counting procedures.

The technologist who seeks information concerning the effect of molds on tomato fruits usually must ponder through a maze of data which relates also to leaves, stems, seedlings and roots. Most of these publications describe also the

physiological, viral and bacterial disorders of the entire tomato plant. Unfortunately also, workers frequently have failed to relate the scientific name of the mold found to the disease of the tomato, therefore, a disease has been known by more than one popular name.

Doolittle (1948) compiled what has been perhaps the most widely referred to list of phyto-pathogens common to tomatoes grown in the United States. Berkely and Richardson (1944) issued a similar summary of fungus diseases common to tomatoes in Canada.

Workers at experimental stations have considered principally the molds on tomatoes which have been responsible for financial losses in individual states. The publications of Young (1946) and Young et al (1940) and of Davis (1948a, b, c, d, e, 1952) are pertinent to this investigation. MacGillivray et al (1950) described symptoms on tomatoes affected by Phytophthora infestans, Alternaria sp., Pleospora sp., Rhizopus sp. and Phytophthora capsici. Beattie et al (1942) listed only Colletotrichum sp. and Alternaria solani as fungi attacking tomato fruits. Linn and Wright (1951) presented valuable data concerning several tomato diseases.

Some investigators have published information concerning only one or a few of the fungus diseases observed or a particular aspect of a tomato disease. For example, Henderson (1942) listed some of the characteristics of early blight and

Phytophthora fruit rot found in Colorado and Heuberger (1949) elucidated the mold problem in Delaware. Middleton and Kendrick (1953) discussed what has been accomplished on tomato disease in California. Cunningham and Lambeth (1951) gave emphasis to tomato disease control.

B. J. Howard (1911) in the Food and Drug Administration of the United States Department of Agriculture introduced a laboratory procedure for counting the number of mold filaments in tomato products. Subsequently the Governments of the United States and of several other countries seemingly have come to regard the Howard mold count as an indication of tomato quality. Howard (1937) gave some of the characteristics of several genera of molds, notably Alternaria sp., Colletotrichum sp., Fusarium sp., Mucor sp., Rhizopus sp., Oidium sp., Penicillium sp., Aspergillus sp., and Botrytis sp. He pointed out that Alternaria, Colletotrichum and Fusarium cause most of the injury to the fruit before the tomatoes leave the field and these molds seldom develop markedly on fruit which have been taken from the field in sound condition.

Eisenberg (1952b) was another worker concerned with the Howard mold count and its application. He listed four molds which commonly attack tomatoes as Alternaria, Colletotrichum, Phytophthora, and Oospora, and described the area affected and the depth of visible rot produced by these molds.

A publication by the American Can Company (1950) reported characteristics observed in the tomato fruit when species of

Alternaria, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Mucor*, *Rhizopus*, *Oidium*, and *Penicillium* were present. It was mentioned also that infection by *Alternaria* could be controlled by using resistant varieties of tomatoes.

In a similar publication Troy (1952) of the Continental Can Company included the following fungi: *Alternaria*, *Colletotrichum*, *Fusarium*, *Mucor*, *Rhizopus*, *Phytophthora*, *Stemphylium* and *Cladosporium*, and gave some of the effects of these molds on tomato fruit.

A limited amount of information has been available in the booklet, *Micro-analysis of Food and Drug Products*, issued by the United States Department of Agriculture (1951).

A summary of the most pertinent information noted in the literature is presented in Table I.

TABLE I

SYMPTOMS PRODUCED IN TOMATO FRUITS WHEN MOLD GROWTH IS PRESENT AS NOTED BY VARIOUS WORKERS

Organism and Common Name of Spot or Disease	Epidemiology	Stage of Fruit Attacked	Symptoms on the Fruit
Alternaria solani (Ell. and G. Martin). Formerly known as Macrosporium solani (Ell. and G. Martin) Early blight Target spot Black rot	Occurs to some extent in most tomato growing regions. Organism can live on decayed plant tissue in the soil. Spores spread by wind, rain, and humans	Enters through stem. Infection starts in sound, small wound or crack. Attacks at the point of attachment to the stem, usually on the top and on the sides, in July	Fruit may drop prematurely from the vines. Sunken black, circular spots, leathery. Affect only the outer fruit tissue. May involve 1/3 area of the fruit. Dark dry decay extends to some depth into the flesh of the fruit. Fungus often completely covers the affected areas appearing as a brownish velvety growth marked with concentric markings.
Alternaria tomato (Cke) Weber Formerly known as Macrosporium tomato Cke. nailhead spot	Fields, transit, storage. Spores spread by wind and rain	At any stage, on any part	Early fruits may show no symptoms when picked but spots develop later. Small, shallow, gray to tan specks. Later specks become 1/16-1/2" diameter with black borders and whitish centers, often a black spot in the white center. Center of spots are slightly sunken. Older spots have centers more definitely sunken and become grayish brown. The surface becomes roughened by drying and tearing back of the epidermis. When numerous spots coalesce, they often cause irregularities on the shape of small fruits. In ripening fruits, tissues around spots remain green in color. Spores produced on the surface of the spots. Fungus ordinarily does not penetrate deeply into the fruit but secondary infections sometimes result.

TABLE I (Cont.)

Organism and Common Name of Spot or Disease	Epidemiology	Stage of Fruit Attacked	Symptoms on the Fruit
Colletotrichum phomoides (Sacc.) Chester anthracnose	Common, widely distributed. Often caused serious losses to canning crops in Atlantic and Central States. Likely to occur on poorly drained soil. Overwinters on tomato refuse. Spores from the spots are splashed to other fruits by rain. Infection appears to come from the soil. Development rapid under favorable conditions as in warm, damp weather.	May attack green fruits but spots do not show until the fruit ripens. Fungus penetrates apparently uninjured fruits. Fruits are ripe or nearly ripe. Apparently the organism does not invade the green fruit under ordinary conditions. Red-ripe, sorted tomatoes left overnight will frequently be badly spotted.	Early - small, circular, inconspicuous, translucent, slightly sunken blemish, 1/8" in diameter. One or more spots on a single fruit. Spots enlarge rapidly on ripe or nearly ripe fruit and reach ultimate size of 1/4 to 1" diameter. These spots soon become depressed and have concentric ring markings. Centers sometimes become tan and show a number of dark specks which are bodies in which spores of the fungus are produced. As spots enlarge the tissues of the fruit collapse. The spots coalesce and cause larger water soaked areas. When the spots reach their limit in size they soften and the entire fruit may rot by the time it is red ripe. Secondary fungi and bacteria may overrun the infection greatly extending it. Fungus frequently penetrates deeply into the flesh. Epidermis remains intact over the lesions. 24-48 hours after a spot appears, center may be dark brown to black in color. Rings are caused by periodic formation of fruiting bodies. During periods of high humidity, cream colored pink or brown spores masses may ooze from the center of the fruit spots. Spores are produced in great numbers.

TABLE I (Cont.)

Organism and Common Name of Spot or Disease	Epidemiology	Stage of Fruit Attacked	Symptoms on the Fruit
Fusarium oxysporium (*F. lycopersici*) Snyder and Hansen	Fungus most active at 80-90°F. Can live almost indefinitely in the soil	Enters through the roots up through the stem. In severely affected plants may pass into the fruit	Fruits not spotted
Phoma destructiva Plowe Phoma rot		Enters through stem cracks or other wounds usually near stem end	Black sunken spots which may bear numerous black pimples containing spores of the fungus
Phytophthora infestans (Mont.) DeBary Late blight	One of the most destructive diseases prevalent in cool (50-80°F) rainy weather. Occurs sporadically. Invades in field and in transit. Infection occurs at any stage of growth. Fruits may seem sound, decay by the time they reach market. Fruits	Usually starts near stem end. Most common on the upper half but may occur elsewhere on the surface	*Early* - Gray green, firm, water soaked spots. Peel has a slightly wrinkled surface with a blotchy, brownish green color. Occasionally show narrow, zonate markings. No definite margins but are usually sunken where decayed and healthy tissues join. Affected area does not ripen. Under moist conditions, a downy white growth of the fungus occasionally appears on the fruit. Other organisms may enter through the blight spots and cause a watery rot.

TABLE I (Cont.)

Organism and Common Name of Spot or Disease	Epidemiology	Stage of Fruit attacked	Symptoms on the Fruit
	decay within a week after infection. Does not seem to survive in dead plant tissue. Spread by wind and rain. Spores by water.		Later - May spread over 1/3-1/2 of a fruit within 2 days. Spreads until much or all of the fruit is affected. Rotted portions remain firm.
Phytophthora parasitica Dast. (P. terrestris Sherb.)	In the field, in transit, in storage. Fungus lives in the soil. Most prevalent in regions subject to prolonged periods of warm, wet weather and in poorly drained soil.	Fungus attacks fruits that touch the soil or those that have soil splashed onto them. Green or ripe fruits may be affected. Organism can penetrate uninjured surface of the fruit at the blossom end.	Early - May show symptoms or little brown dots at picking. Green fruits do not become soft for some time after infection. First symptom is a small or grayish green or brown water soaked spot. Spots enlarge rapidly in warm weather. May cover half or more of the fruit. May have no definite markings but usually show darker zonate bands that give the disease the name, buckeye rot. However, fruits infected with Phytophthora infestans occasionally show similar markings. Surface of the spot is firm, has a smooth but not sharply defined margin, in contrast to late blight rot whose spots have a roughened surface and are slightly sunken at the margins. Rot is usually leathery in consistency and usually affects the fruit to a considerable depth. When rot develops slowly, definite zonations appear. In wet weather the grayish white fungus growth may develop on the rotted fruit.

TABLE I (Cont.)

Organism and Common Name of Spot of Disease	Epidemiology	Stage of Fruit Attacked	Symptoms on the Fruit
Rhizoctonia solani Kahn Soil rot *Rhizoctonia* rot	In field, transit, and storage. Organism is likely to be present in soil wherever tomatoes are grown in the field. Most common in rainy weather when top-soil remains wet and on low, poorly drained soil.	Green or ripe fruit. May penetrate through unbroken epidermis or through wounds. Occurs on surface of fruit in contact with soil or where soil splashed onto fruit.	Early - May show no symptoms or only traces of brown. Spots have alternating light brown and dark brown concentric rings. Rotted areas become 1/4-1" in diameter, have circular or irregular shape. Spots have sharply outlined zonate markings, narrower and closer together than those of buckeye rot. Later - Spots enlarge gradually and may become 1" in diameter and markings tend to become dark brown and centers frequently break open. Rupturing of the surface and narrower and sharper markings distinguish disease from buckeye rot. Brown mold with sclerotia may develop in the cracks. Fruits touching wet soil sometimes show a surface layer of white mold and spores.
Rhizopus sp. *Rhizopus* rot	On ripening or ripe tomatoes in the field, transit, storage. The organism is the common bread mold	Enters nearly mature fruits through wounds	Early - Start as blister-like areas. In a few hours destroy the entire fruit. Rotting fruits often bear large masses of the fungus with black fruiting bodies or the fruit may be changed into water bags without external mold.

TABLE I (Cont.)

Organism and Common Name of Spot or Disease	Epidemiology	Stage of Fruit Attacked	Symptoms on the Fruit
Sclerotium rolfsii sacc. Southern blight	Occurs to some extent in southern United States. Comparatively rare in other tomato growing regions. Apparently no spores. Spread through soil. Infected areas widen from year to year. Also spread by sclerotia which can live in the soil for sometime. Spread by rain and soil cultivation. Makes little growth below 68°F. Requires abundant moisture. Most prevalent on light, poorly drained soil.	Where fruit touch soil	Yellowed slightly sunken areas that break open as the spots enlarge. Fruit soon collapses and is covered by a growth of the fungus.
Septoria lycopersici speg. *Septoria* blight or *Septoria* leaf spot	Rarely infects fruit		

Experimental and results

Studies of Field Tomatoes

At the beginning of the 1953 tomato canning season a laboratory was established at the Fettig Tomato Canning Company, Elwood, Indiana. This was centrally located in the heart of the tomato growing region and permitted samples to be taken from a broad area. Tomatoes which had been grown for canning were collected from fields as shown in Figure 1. A total of 27 fields were surveyed. Thus at some of the points indicated as many as four fields were examined at different times during the season.

An effort was made to obtain tomatoes which would yield as many types of mold growth as possible. Generally 30-40 tomatoes were picked from each field and of these the ten most likely to yield different genera and species of molds were selected. The tomatoes were uniform in size, weighed between 100-200 grams, were ripe, red in color and were still attached to the vines. Conceivably many might have been picked for canning purposes at the time. As over 80 percent of the tomatoes grown for canning in the areas studied were of the Rutgers variety, this variety was chosen unless otherwise specified.

The tomatoes were placed in individual ventilated boxes and transported within a few (less than 5) hours to the laboratory where they were examined immediately.

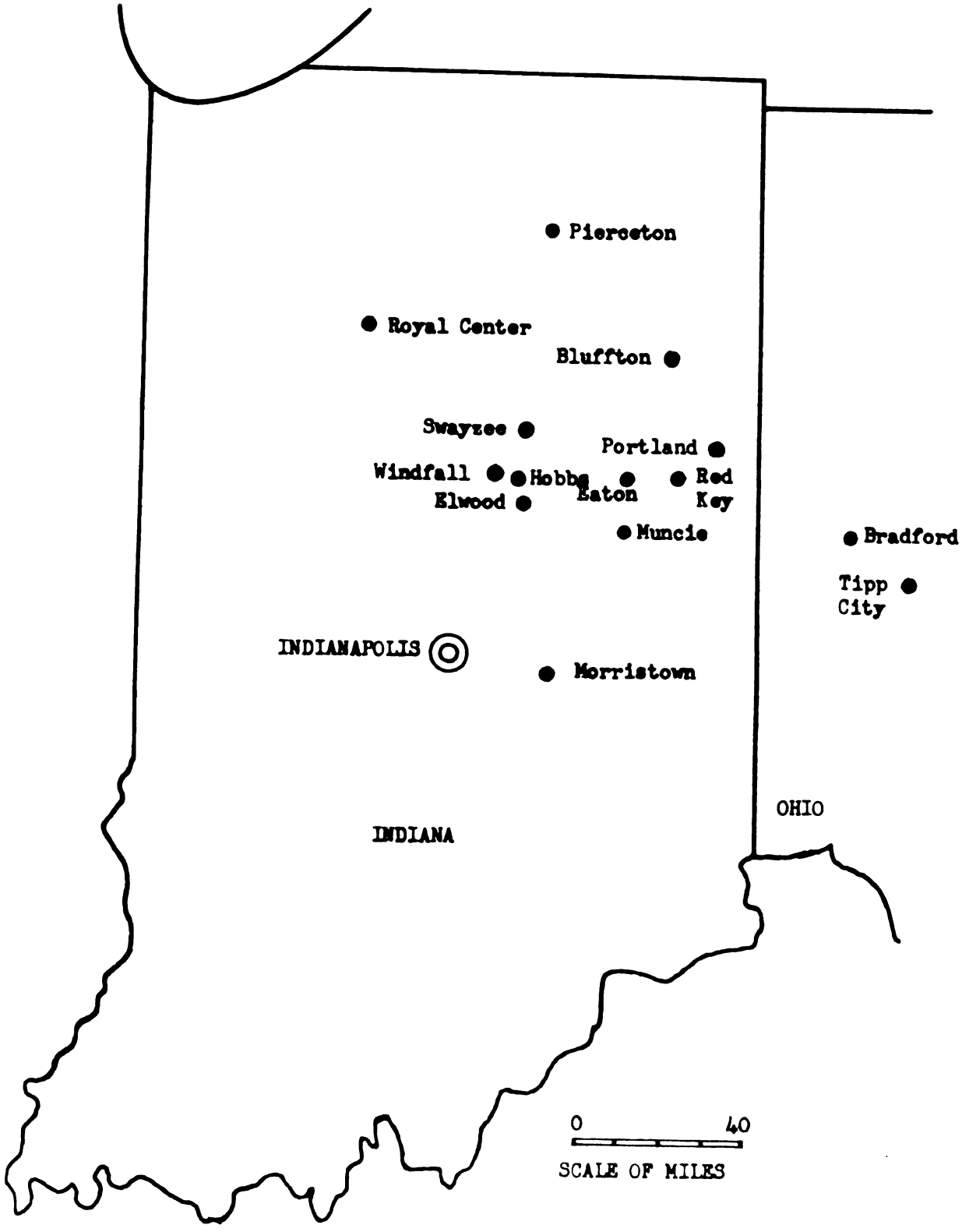


Fig. 1. Location of tomato sampling points in the States of Indiana and Ohio.

The extent of the macroscopic tissue change was measured or estimated in relation to the whole tomato fruit. The presence of external mold was determined by observations employing a 10X objective. The type of mold which was thought to be present was noted. Two cultures were made from the surface growth when this was evident and, in addition, two pieces were cut aseptically from any damaged tissues and cultured by placing these onto Petri plates containing potato dextrose agar (Difco). As the molds developed they were identified when possible or transferred onto potato dextrose agar slants for identification studies later in the year.

Many of the tomatoes obtained from the fields contained molds which were isolated as pure cultures. Thus it was possible to group the gross characteristic symptoms produced in tomatoes by these molds (Table II) .

Considerable variation was observed in the extent and type of damage which occurred in tomatoes when mold growth was present. The extent and type of damage was shown to be related to the genus of mold present. Molds such as Mycelia sterilia, Trichoderma sp. and Hormodendrum sp. which usually have not been mentioned in connection with tomatoes were found. Species of Oospora, Fusarium, Mucor, Rhizopus and Rhizoctonia produced soft rots which frequently consumed a large segment or almost all of the tomatoes.

By contrast, Alternaria sp. and Colletotrichum sp. frequently produced only superficial markings and damage to the

TABLE II

GROSS CHARACTERISTICS OF THE TOMATO FRUITS OBTAINED DURING FIELD STUDIES

Organism Isolated	Tomato	Appearance of the Tomato
Alternaria	A	5 splits, black
	B	1" soft spot, dark center
	C	1-1/2" black spot
	D	3/4" dry, black brown sunken spot
	E	cracks at top
	F	1" dry, round, sunken spot
	G	black cracks at stem end
	H	cracks at stem end
	I	3 wide splits in ripe firm tomato, covered with mold
	J	1/3 sun scald, covered with fungus growth
	K	3 cracks, 1" diameter
	L	2 black, hard, sunken spots, 1" diameter
	M	sunscald on 2-1/2" diameter with black markings
	N	1-1/4" diameter sun scald area with black markings
	O	black sunken area on 2 x 1/2" sun scald area
	P	2" sun scald area, with black growth in this
	Q	4 splits, 1/2 x 1" with Alternaria
	R	growth cracks with black mold in these
	S	growth cracks with
	T	1/2" black spot in middle of sound fruit
	U	3 splits with Alternaria, soft spots 1" diameter around splits
	V	1-1/2" x 2" sun scald with Alternaria on surface
	W	2" rot, hard on surface, also split 3/8 x 3/8
	X	sunscald with Alternaria
Colletotrichum	A	dark brown to black, faint concentric rings in sunken soft area 1/2" diameter
	B	5 tan brown spots about 3/8" diameter
	C	1/3 soft, 12 spots 1/3" diameter, sunken light and dark concentric rings
	D	6 spots, sunken, light and dark rings darker in center spot

TABLE II (Cont.)

Organism Isolated	Tomato	Appearance of the Tomato
Colletotrichum	E	4 spots, sunken, firm, black smooth surface
	F	2 sunken spots, dark and light rings
	G	1/2 rotted, dried black surface
	H	3-1/2" brown spots
	I	15 spots 1/4 to 1/2" diameter
	J	3/4" black, sunken spot
	K	3/4" soft spot
	L	firm, brown area, no surface mold
	M	1/2" spot on ripe, firm fruit
	N	1/3" sun scald, 2-1/2" diameter with 3 small 3/8" sunken spots on the sun area
	O	1/4" sunken spot, 6 in number
	P	15 spots, 4 of these large 1" diameter
	Q	9 spots, 3/8 x 1" diameter (Stokes)
	R	15-20 spots fused together to make up 2" diameter spot (Stokes)
	S	1/2 soft rot
Fusarium	A	1/3 soft rot, insect injury, white hyphae on surface
	B	nearly all soft rot, pink white hyphae
	C	1/2 soft rot, in two large holes, white-pink hyphae in holes
	D	1/2" hold with 1" soft area
	E	1/2" soft spot with firm skin
	F	1/2 soft rot 2" in diameter
	G	1" rotted spot, black, pink-brown surface mold
	H	cracks at stem end, pinkish-orange mold, some shrivelling
	I	cracks at stem end
	J	1" round, soft spot covered with grayish yellow mold
	K	1-1/2" soft spot
	L	black cracks at stem end
	M	2/3 soft rot, gray brown mold
	N	1" soft rot
	O	1/2" deep, insect injury - soft rot
	P	1/2 soft rot at blossom end, with deep crack
	Q	1/2" deep, insect injury, soft spot

TABLE II (Cont.)

Organism Isolated	Tomato	Appearance of the Tomato
Fusarium	R	deep, 1/2" insect injury - soft rot
	S	1/2 soft rot at blossom end with deep crack
	T	1/2" deep, insect injury, soft spot
	U	2 holes near stem, soft spot 1 1/2"
	V	very soft with 5 cracks
	W	somewhat soft with 3/4" dried shrivelled spots
	X	2" soft rot area on side near split
	Y	1-1/2" area soft rot
Mucor	A	1/2 soft, 1/2 rotted
	B	2" split on side, 3/8" wide
	C	1/3 soft rot, 1-3/4" diameter
	D	soft, dark red spot 1" diameter
	E	3/4" soft rot, dark red color
Mycelia sterilia	A	3-1/4" spots, light brown 2" soft rot area with 4 spots in the middle (Stokes)
Oospora	A	1" soft spot, skin broken
	B	1-1/2" brown area, concentric rings, split
	C	wide crack across 1/2 of fruit, soft area
	D	2 x 3" rot entailing 1/3 of fruit, soft mushy, no color change (Stokes)
	E	1" soft spot, crack with black growth on top, white underneath (Stokes)
Penicillium	A	1/4" soft spot

TABLE II (Cont.)

Organism Isolated	Tomato	Appearance of the Tomato
Phytophthora	A	2" spot on side, 1" spot on other side
	B	2 adjoining spots, 3" x 1-1/2"
	C	2" spot and 1" spot
	D	1-1/2" spot
	E	3 fused spots, total diameter 2-1/2"
	F	2 2 x 1" areas on sides, entailing about 2/5 fruit
	G	green fruit, 4/5 blighted mostly firm green color, some orange, 2 spots 1-1/2"
Rhizoctonia	A	appearance like soil, rot 1/3 tomato, soft
	B	1/3 soft spot, brown ring in center, surrounded by yellow and green concentric rings
	C	2" soft spot
	D	2/3 soft rot, water soaked
	E	1/2" spot, soft green-yellow
	F	1/2 soft rot, green center, small concentric brown rings
	G	1/4 area on blossom end, dark red water soaked, soft
	H	dark black spots, 3/8" green edges, spots firm, fine thin threads in spots
	I	1/2 soft rot, few vague, brown concentric rings
	J	1-3/4" soft brown rot, 1/4 of tomato green
	K	1/2 soft rot 2" spot in center
	L	2" narrow split, 2-1/2" soft brownish area
	M	1-1/2" brownish soft area, concentric rings
	N	1" insect damaged spot covered with black growth
	O	1/3 area rotted, dark green
	P	1/3 soft rot, 2" area
	Q	1/3 soft rot, also cracked
	R	1/2 soft rot, no surface growth
	T	3/4 soft rot, with greenish cast

TABLE II (Cont.)

Organism Isolated	Tomato	Appearance of the Tomato
Rhizoctonia	U	1-1/2" rot, concentric rings
	V	3 spots about 3/4" diameter
	W	1" soft spot, greenish to black
	X	1" spot, brown rings
	Y	reddish, black, soft spot, 1" diameter
	Z	2 dark red spots, concentric rings, 1-1/4" diameter
Rhizoctonia	A (1)	soft brown rot with crack
	(2)	soft red rot, no surface break
	(3)	2-1/2" diameter soft spot entailing about 1/2 fruit
	(4)	2" soft rot, dark red in color
	(5)	2" soft reddish brown rot, small split with white hyphae
	(6)	2" dark red spot with crack containing white hyphae
	(7)	1-1/2" soft rot on end and side, dark red rot
	(8)	1/3 soft rot, greyish, grey-brown hyphae on surface
	(9)	2/3 soft, water soaked, green area in rot
Rhizopus	A	black spot 1" diameter, hard near stem end
	B	2/3 soft rot, skin punctured in one spot (Stokes)
	C	1/2 soft rot with cracks
	D	cracks at stem end with white fungus, 2" soft area

tomatoes. Areas invaded by Alternaria were distinctly visible, firm and black. Colletotrichum growth was observed in many of the tomatoes seen in the fields. Representative tomatoes often showed the presence of only minute spots, one to a dozen or more on a single tomato. These spots extended into the tomatoes for a distance of 1/8 to 3/8 in. and appeared as bruised or water soaked areas.

It was not uncommon to find a mold with much aerial hyphae on a broken tomato. When this mold growth was removed little or no damage was apparent.

Macroscopic symptoms evidenced by many of the molds growing on tomato fruits are shown in Figure 2. Attempts to identify the genera of the molds present without the aid of cultural findings frequently proved unreliable even when the provisional identification was conducted by experienced mycologists. This proved particularly true when a number of molds were present in the rotted areas of the fruit.

Laboratory Inoculation of Tomato Fruits with Molds

Sound Rutger tomato fruits which had been picked in the State of Indiana were sorted carefully to obtain tomatoes free from blemishes and mold growth. These tomatoes were divided arbitrarily into three groups:

A = green; B = half ripened; and C = fully red ripened.

Alternaria solani (on sun scald)

Alternaria solani (in cracks)

Alternaria solani (with Cladosporium sp. or Rhizopus)

Alternaria and Colletotrichum phomoides

Colletotrichum phomoides

Fusarium oxysporium

Fig. 2. Macroscopic characteristics produced on tomato fruits by some of the principal molds under field conditions.

Oospora variabilis

Penicillium sp. with Fusarium oxysporium

Phytophthora infestans

Rhizoctonia solani

Fig. 2. (Continued) Macroscopic characteristics produced on tomato fruits by some of the principal molds under field conditions.

The fruits were immersed for one minute into a bath consisting of equal parts of a solution of hypochlorite and ethanol and then given two washes in sterile distilled water. Two tomatoes from each of the A, B and C groups were inoculated by a single needle puncture with molds which had been isolated from tomatoes obtained during the first part of this study. Thirty-two cultures, representing 11 genera were employed.

The tomatoes were placed in individual, sterile, one quart screw-capped jars. The metal lids of these jars had been replaced with thin layers of absorbent cotton in order to prevent contamination while allowing adequate exchange of gases during mold development. A uniformly small amount of distilled water was added to each jar intermittently during the test period.

A duplicate set of tomatoes from groups A, B and C, treated and sterilized in the same manner as just described, was placed also in jars. However, instead of needle puncture, spore and vegetative suspensions were placed on the surface of the tomatoes. The suspensions had been prepared by adding sterile physiological saline solution to the surface of two week old cultures and removing the surface growths with an inoculating needle.

All sets of tomatoes inoculated with molds in the laboratory showed blemishes after 48 hours (Table III). However, some of these blemishes were very slight.

At the end of 4 days tomatoes inoculated with Botrytis, Mucor, Oospora and Rhizopus and, to a lesser degree, Penicillium and Aspergillus had areas evidencing decomposition (Figures 3 and 4). Some of these areas were regular circles particularly those produced by Oospora, Penicillium and Aspergillus. After nine days tomatoes invaded by Botrytis, Fusarium, Mucor, Oospora and Rhizopus were rotten (Table III).

In contrast to the other molds, only minor blemishes were produced by Alternaria solani, Colletotrichum, Hormodendrum, Trichoderma and Penicillium at the end of two days incubation and the results after 9 days were not substantially different (Table III). The blemishes as they appeared at the end of 4 days are shown in Figure 4.

It was interesting to note that Rhizopus produced cracks in tomatoes not only at the point of inoculation but also for some distance ahead (Figure 3).

No consistent variation in attack was noted among the tomatoes in the three stages of ripeness although tomatoes in the green stage frequently retained greenish areas at the point of inoculation.

There was no apparent invasion of the tomatoes which had been treated and inoculated, without puncturing, on the surface with molds. Nor was any growth detected on the surface of these tomatoes.

TABLE III

EFFECT OF EXPERIMENTAL INOCULATIONS OF WHOLE TOMATO FRUITS

Organism	Source	Appearance at 2 days	Appearance at 9 days
Alternaria	1E	C - No change	No sunken area. Black 1/4" diam.
		B - 1/4" diam.	No sunken area. Black 1/4" diam.
		A - No change	No visual mycelium. Black 1/2" diam. surrounded by flat zone
	5A	C - 1/16" area	
		B - No change	1/2" flat area with black central zone
		A - 1/16" zrea	1/2" flat black area with outside zone also flat
	9E	C - 1/4" sunken area	1/3" diam. light dark area
		B - No change	1/3" diam. light dark area
		A - 1/16" area	1/4" dark central area, about 1-1/2" soft brown surface rot
Aspergillus	2B	C - 1/4" area covered with mold	Approx. 1-1/2" area decomposed with heavy growth at point of inoc.
		B - 1/4" area covered with mold	" " " " "
		A - No change	" " " " "
	4B	C - No change	ca. 1/2-1" decomp. very sl. growth
		B - 1/16" pit	" "
		A - No change	" "
	28	C - 1/4" flat area	Sunken decomposed area ca. 1-1/2" diam. with heavy black growth
		B - 1/4" flat area	" "
		A - 1/4" flat area	" "

TABLE III (Cont.)

Organism	Source	Appearance at 2 days	Appearance at 9 days
Botrytis	4F	C - No change	Soft, but not watery
		B - 1/8" flat area	Watery and decomposed
		A - No change	Firm, no change
	3C	C - 1/8" area with growth	Decomposed and watery
		B - 1/8" area with growth	Decomposed and watery
		A - 1/2" area with growth	Decomposed and watery
	3E	C - 1/8" flat area	Watery and decomposed
		B - 1/4" flat area	Watery and decomposed
		A - No change	Firm, no change
Colletotrichum	4E	C - 1/32" area affected	1/4" area affected. No blackening except pin point at site of inoc.
		B - No change	1/8" dark area surrounded by 5/8" flattened, light brown area
		A - 1/32" area affected	Same as C above but area about black pin point was clear green
	10J	C - 1/8" pit	Dark 1/8" area at point of inoc. surrounded by 1/4" green area. Fruit beginning to break down
		B - 1/8" pit	Dark 1/4" area, split open. 1-1/4" of rot, flat
		A - 1/8" pit	Dark 1/8" area at point of inoc. surrounded by 1/8" green zone. Rest of fruit red ripe
	18H	C - 1/8" pit	Black 1/8" area at point of inoc. surrounded by 1-1/2" dark brown, flat area
		B - No change	Black 1/8" area at point of inoc. swollen up, surrounded by 1-14" brown area

TABLE III (Cont.)

Organism	Source	Appearance at 2 days	Appearance at 9 days
Colletotrichum		A - No change	Black 1/8" area at point of inoc. surrounded by 1/8" green zone. Rest of tomato ripe
Fusarium	2A	C - mold, no pitting	Entirely decomposed and watery
		B - mold, no pitting	Entirely decomposed and watery
		A - mold, 1/32" pit	1/4" black area, not flattened
	5E	C - 1/32" pit	Pin point growth at point of inoc. surrounded by 1/2" light pink area, in contrast to red of fruit elsewhere
		B - 1/16" pit	Pin point growth at point of inoc. surrounded by 1-1/2" dark, decomposed area
		A - No pit, but heavy mold growth	Watery and somewhat decomposed
	14B	C - 1/16" pit	1/4" pit covered with white mold
		B - 1/8" pit	1/8" area surrounded by 1-1/2" dark area, not sunken
		A - 1/4" pit	Heavy white growth at point of inoc. surrounded by 1/4" zone with dark ring
Mucor	1B	C - 1/8" dark zone	1/8" black area surrounded by 1/8" brown area
		B - 1/8" dark area	1/8" black area surrounded by 1/4" yellowish green area
		A - 1/4" dark area	1/8" black area surrounded by 1/8" brown area

TABLE III (Cont.)

Organism	Source	Appearance at 2 days	Appearance at 9 days
Mucor (Cont.)	1G	C - 1/2"	Fruit split in different directions, white mold growth
		B - 1/2" ring, mold in middle	Fruit split in different directions, white mold growth
		A - 1/4" dark ring	1/16" green area surrounded by 1/16" yellowish area. Rest of fruit ripe
	13F	C - 1/2" dark area	Very heavy growth at point of inoc. No obvious breakdown. Growth ran over surface
		B - 1/4" dark area	Very heavy growth at point of inoc. No obvious breakdown. Growth ran over surface
		A - 1/4" dark area	Growth (white) over tomato, partly covering it.
Oospora	1F	C - 1/4" rot	Contaminated
		B - some mold	Very heavy raised white growth, watery, cracked fruit
		A - 1/2" flat area, some mold	Very heavy raised white growth, watery, cracked fruit
	12H	C - 1/2" dark, flat ring	Very heavy raised white growth, watery, cracked fruit
		B - 1/2" dark, flat ring	Contaminated
		A - 1/2" dark, flat ring	Very heavy raised white growth, watery, cracked fruit
	16F	C - 1/2" dark, flat ring	1/2 of fruit soft, no growth apparent
		B - 1/2" dark, flat ring	1/2 of fruit soft, no growth apparent
		A - 1/2" dark, flat ring	1/2 of fruit soft, no growth apparent

TABLE III (Cont.)

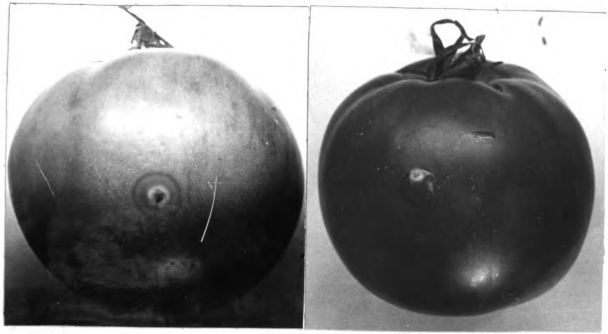
Organism	Source	Appearance at 2 days	Appearance at 9 days
Penicillium	17T	C - 1/4" flat area	1/4" gray, green white growth surrounded by 2" wrinkled, brown area
		B - 1/2" flat area	1/2" greenish white growth
		A - No change	1/8" raised white growth surrounded by 3/4" brown, flat area
	2B	C - 1/32" area	1/32" area surrounded by 1-1/4" brown area. No tufts of mycelia visible
		B - No change	1/32" white area surrounded by 1/2" brown area
		A - No change	1/32" white area surrounded by 1-1/2" dark, brown area
	11I	C - 1/32" pit	1/4" gray, green white growth, surrounded by 2" wrinkled, brown area
		B - 1/16" pit	1/2" greenish white growth surrounded by 2" brown, sunken area
		A - 1/32" pit	1/8" raised white growth surrounded by 3/4" brown flat area
Rhizoctonia	13A	C - No change	No rot, no obvious growth
		B - No change	Contaminated
		A - 1/8" pit	No obvious change except 1/8" green area at point of inoc.
	15C	C - 1/8" pit	1/16" white growth, surrounded by 1" rotten area
		B - 1/16" pit	1/4" raised growth, irregular, surrounded by 1" brown rotten area
		A - 1/16" pit	1/4" raised growth, irregular, surrounded by 1" brown rotten area

TABLE III (Cont.)

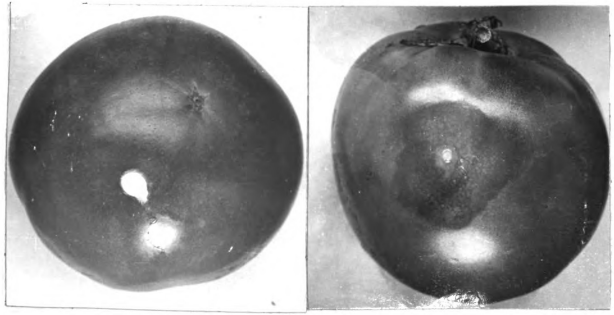
Organism	Source	Appearance at 2 days	Appearance at 9 days
Rhizoctonia (Cont.)	9A	C - No change	1/8" white growth, surrounded by 1/2" rotten area
		B - No change	No change
		A - 1/8" discoloration	1/2" dark area with 1/16" white center
Rhizopus	9G1	C - 1" discoloration	Watery and decomposed
		B - 1-1/2" flat, discolored area	Watery and decomposed
		A - 1" flat, discolored area	Watery and decomposed
	3F	C - 1" dark area	Watery and decomposed
		B - 2" dark area some growth	Watery and decomposed
		A - 1" dark area some growth	Watery and decomposed
	1C1	C - 2" dark area	Watery and decomposed
		B - 1" dark area	Watery and decomposed
		A - 1" dark area, much growth	Watery and decomposed
Trichoderma	2D	C - No change	Dark area, 1-1/4" diameter
		B - No change	Black area, 1/8" diameter
		A - 1/8" dark area	Black area, 1/8" diameter

TABLE III (Cont.)

Organism	Source	Appearance at 2 days	Appearance at 9 days
Trichoderma (Cont.)	4I	C - No change	1/4" dark area, surrounded by 1/4" green area, rest of fruit red
		B - 1/16" dark area	1/4" dark area, surrounded by 1/4" green area, rest of fruit red
		A - 1/16" dark area	1/4" dark area, surrounded by indef. brown area ca. 1/8" diameter

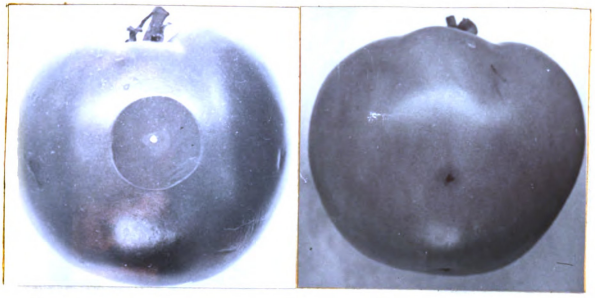


Aspergillus Botrytis



Fusarium Mucor

Fig. 3. Effect of experimental inoculation of tomatoes with Aspergillus, Botrytis, Fusarium, Mucor, Oospora, Rhizoctonia, and Rhizopus (after 4 days at room temperature).

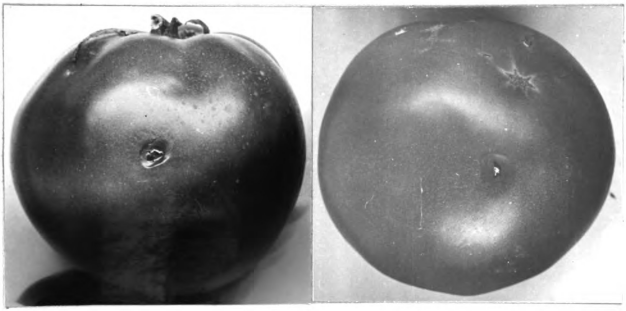


Oospora

Rhizoctonia

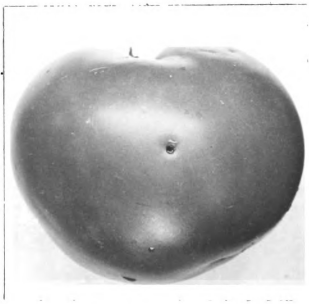
Rhizopus

Fig. 3. (Continued) Effect of experimental inoculation of tomatoes with Aspergillus, Botrytis, Fusarium, Mucor, Oospora, Rhizoctonia, and Rhizopus (after 4 days at room temperature).



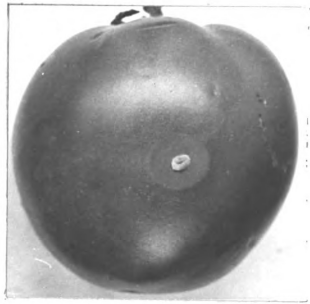
Alternaria

Colletotrichum

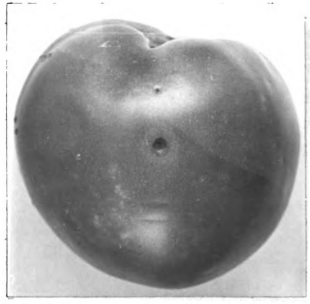


Hormodendrum

Fig. 4. Effect of experimental inoculation of tomatoes with Alternaria, Colletotrichum, Hormodendrum, Penicillium and Trichoderma (after 4 days at room temperature).



Penicillium



Trichoderma

Fig. 4. (Continued) Effect of experimental inoculation of tomatoes with Alternaria, Colletotrichum, Hormodendrum, Penicillium and Trichoderma (after 4 days at room temperature).

Discussion

This study has shown that considerable training in mycology is necessary to identify the types of molds which may be present on tomatoes. Cultural and microscopical examinations frequently are necessary for positive identification of these molds. The genus, species and strain determines whether appreciable damage is done to the tomato fruit concerned. In addition, the variation in the severity of attack would be influenced by many factors, such as the variety of the tomato, soil and weather conditions existing in certain areas and spray programs. The rapidity of handling the tomatoes after they reach the factory is also very important. Excessive humidity promotes mold growth (Block, 1953). Yet, unless the relative humidity is high, tomato fruits will have excessive loss of moisture to the surrounding air and in time most of them will become wilted and shrivelled. Rose et al. (1949) reported that ripe tomatoes at 60°F evolve approximately 5640 BTU per ton per 24 hours when stored.

The Howard mold count makes no allowance for the significance of the types of molds present and, in any event, the average mold counter would not have the experience to identify the types likely to be encountered. It is obvious, however, that it would not be reasonable to regard all molds as being of equal importance in causing rot in tomatoes. It has been

observed that molds in the fields and in the factory produced characteristic changes in tomato fruits, some severe, some minor.

It was interesting to observe that some strains of Rhizopus produced cracks in tomatoes. It appears that even a minute perforation of the tomato cell wall which allows the introduction of this organism may be the precursor of extensive damage. No reference to this action has been noted in the literature. Instead, it has been mentioned that molds grow in cracks; and that the cause of cracks in tomatoes has been obscure. It was suggested that soil and weather conditions may be involved (Howard, 1937).

It was interesting to note also that under experimental conditions while many of the molds produced irregular lesions in tomatoes, strains of Aspergillus, Colletotrichum, Oospora and Penicillium appeared to produce a metabolite which was radiated evenly throughout the tissue to give circular lesions.

It can be appreciated that considerable difficulty would be experienced by a canner when many of his incoming tomatoes showed Colletotrichum phomoides spots. The number of these blemishes on a single tomato were shown to be numerous on occasion, and would involve extensive trimming which would substantially increase costs of production. Another consideration is that in the early stages of attack by this organism relatively little damage appeared obvious in the tomatoes and

might be overlooked by housewives commonly employed in trimming and sorting in the factory. The appearance of these spots on the canners stock would depend on soil and weather conditions in his particular area and might be sufficient to force him out of business when he is in the position of competing with processors in other areas relatively free from the effects of this organism. Other organisms may present a similar problem. For example, Alternaria often was apparent only as a small brown or black area not unlike a scar at the stem end of the tomatoes. It would not appear reasonable to always interpret the mold filaments present as an indication of a rotten condition in the tomatoes. It was interesting to note that similar blemishes were observed on prize tomatoes on exhibition at a state agricultural fair.

There appear to be many conditions which may influence the development of molds.

Brown (1922) showed that certain volatile substances given off from plant tissues, especially when the latter are bruised have a distinct effect on the germination of fungal spores. Distinct stimulation to Botrytis was provided by apples but depressed by potatoes. This stimulative effect was more readily discernible when the spores were of feeble germinative capacity.

Later Brown (1948) reported that if a host is held at warm temperatures, resistance could yield to susceptibility and that pathogenicity of fungi may be greatly influenced by varied illumination, manuring or even the presence of another parasite in the tissue. He emphasized that "stalling substances" produced by a fungus may inhibit its growth.

PART II

MOLD COUNTS, FLAVOR AND pH CHANGES IN RELATION TO STRAIN OF MOLD AND PERCENTAGE ROT PRESENT IN TOMATOES

Introduction

Following a packing season many canners have been confronted with Government seizures of their processed tomato products. Frequently the basis for these seizures has been that the Howard mold count of certain packs has exceeded tolerances established by the Food and Drug Administration.

Many packers have claimed that it has been almost impossible to meet these tolerances under certain conditions. Some processors have claimed that when a mold is present on fresh tomatoes, the fruit sometimes may have superior flavor and odor without any apparent loss in quality in other respects.

This study was undertaken to show what relationship exists between the percentage of fruit showing mold and Howard mold counts. It was thought desirable to investigate also, what influence, if any, the growth of various molds has on the acidity of tomato fruit and what changes in flavor and odor result when molds are present in tomatoes and in tomato juice.

It has been shown elsewhere in this study that molds may produce only minor blemishes on tomato fruits.

Review of Literature

Relationship between Mold Count and Percentage Visible Rot in Tomato Fruit

After the passage of the first Pure Food Law in 1906, in response to numerous requests from industry as to how to test tomato products and interpret the results, the Howard mold count technique was described for the first time (Howard, 1911).

In 1913, using the principles of the Howard mold count method, regulatory actions against canners were instituted but it was not until 1916, however, that the first mold count tolerance was announced. This was for comminuted tomato products and allowed 66 percent positive fields (Smith, 1952).

In June, 1917, some of the more important points covering sanitary control of tomato plants were stressed (Howard and Stephenson, 1917a).

In October, 1917, again in response to many requests for definite details of the test, more information concerning the mold count method was given with a few minor changes (Howard and Stephenson, 1917b). It was stated that very few tests had been made to correlate the microscopic counts with the amount of rot by weight when the Howard mold count had

been first introduced and data on this aspect was presented. Tests were conducted both on laboratory prepared and plant samples. Despite the fact it was somewhat difficult to prepare in the laboratory a pulp of just the same texture as that made under good factory conditions, the same general relationships between the percentages, and mold counts were noted. No laboratory samples with less than 5.5 percent rot gave a mold count of more than 50. No factory samples with less than about 4 percent rot gave a count of more than 60. In this same report it was claimed that no high mold counts occurred in samples low in amount of rot but that low counts were obtained in samples containing a substantial amount of rot. Reference was made to the fact that when stock is properly handled the mold count is of greater importance than the counts of bacteria and yeasts in judging the condition of the raw stock. Bacteria and yeast tolerances were given also in this report.

Darling (1922) criticized the Howard mold count and as a result of many complaints concerning the technique conducted an investigation under the auspices of the National Research Council for the Society of American Bacteriologists. He contended that the kind of fungus is important and that some hyphae break apart more readily in some instances than in others. Darling concluded that the Howard method for determining molds on tomato products could not be depended upon

to give very accurate results. He referred also to sample variability, the type of fungi and the error introduced by different analysts.

Later, Howard (1937) listed seven types of defective tomatoes most frequently described.

The first Government standard for tomato juice was announced on July 1, 1936. This allowed 35 percent positive mold count fields. Subsequently on July 27, 1938, it was reduced to 25 percent and on June 17, 1940 further reduced to 15 percent. On January 13, 1941, the tolerance was raised to 20 percent (Smith, 1952).

Eisenberg (1952a), Chief of the Microanalytical Branch of the Federal Food and Drug Administration, emphasized that low mold counts could be found even when substantial amounts of rotten tomatoes were employed. He mentioned particularly tomatoes infected with late blight (Phytophthora infestans) but indicated that filaments of this mold in some respects do not meet the empirical rules laid down for guiding mold counters and that there is reason to believe that low counts from such tomatoes may be due to failure to recognize the filaments of this mold.

In another publication, Eisenberg (1952b) reported counts from samples prepared in the laboratory from tomatoes showing various molds. Some of his data is of interest and is presented here (Table IV).

TABLE IV

MOLD COUNT IN RELATION TO PERCENT VISIBLE ROT IN WHOLE TOMATOES

Taken from Eisenberg (1952b). Partial data only.

Organism	Visible Rot	Mold Count
Alternaria	2.7	53
"	2.5	39
"	6.9	94
"	10.1	79
"	3.1	61
Colletotrichum	0.3	0
"	0.4	5
"	7.0	92
"	0.6	18
"	5.1	43
"	5.9	57
"	3.1	55
"	0.6	13
"	1.1	20
Oospora	1.9	55
"	9.9	60
"	18.0	62
"	14.6	89
"	10.0	100

Many articles concerning the application of the Howard method to tomato products have appeared in trade publications (Eisenberg, 1952a; Jones, 1940; Jones and Ferguson, 1949; Jones and Pierce, 1947; Gould, 1952 and 1953; Siegel, 1954; Siegel and Strasburger, 1951; Troy, 1950). These contributions usually have stressed the desirability of keeping mold counts within the Government tolerances and have been critical of canners whose packs have exceeded these tolerances.

Smith (1948a) listed Mucor, Aspergillus, Penicillium, Oospora, and Colletotrichum as examples of molds found in mold counts but did not describe the effect of these molds on tomatoes. Later he stressed sorting and trimming procedures and presented data showing the distribution of mold filaments in tomato tissue (Smith, 1948 b).

Influence of Mold Growth on Flavor, Color and Odor of Tomato Juice

Culler et al. (1949) believed that while mold counts have been adopted as a measure of quality, more should be known about the actual effects of these microorganisms on the products. These workers tested, in tomato juice representative strains of Alternaria, Aspergillus, Colletotrichum, Fusarium, Mucor, Penicillium, Rhizoctonia, Rhizopus and Trichoderma in addition to other fungi. They examined the pH, color, taste, odor, and also changes in the refractive

indices. They reported that no off-odors or flavors were detected in the tomato juice after incubation at room temperature for one week but that marked pH changes did occur.

Kertesz and Loconti (1944) investigating consistency said that little appears to have been known about the chemistry and physiology of flavor in tomato juice except that it is influenced by the proportions of sugar and acid in the juice.

Experimental and Results

Relationship between Mold Count of Tomatoes and Percentage Visible Rot

Tomatoes showing the principle types of molds known to be present in Indiana and Ohio fruits as found in Part I of this study were selected from the factory platforms and from the fields. These tomatoes were red-ripe unless otherwise noted. The decomposed section in each tomato was cut out, weighed and expressed as a percentage of the weight of the individual whole fruit. The affected area was combined with the balance of the whole tomato and passed through a laboratory cyclone containing a 0.027 screen.*

Mold counts were made employing the method advocated by the Association of Official Agricultural Chemists (1950).

*Manufactured by the Cephaly Experimental Company, Brentwood, Maryland.

Confirmation of the type of mold present was made by culturing. The results are given in Table V.

A Howard mold count of 100 was obtained when the percentage of rot present in the sample was as low as 1.9 percent (Table V). Counts in the range of 50-100 percent were noted frequently when the amount of rot present was less than 2 percent. This applied regardless of the genus of mold present. In one instance Alternaria when present initially to the extent of producing only 0.1 percent visible rot later gave a Howard count of 57.

The high Howard mold counts obtained in this study when low concentrations of visible rot were present are in contrast to those results presented by Howard and Stephenson (1917b), who noted that no laboratory samples with less than 5.5 percent rot gave a mold count of more than 50 and that no high mold counts occurred in samples containing only a small amount of rot.

The emphasis in published works (Eisenberg, 1952a; Smith, 1952) has been that a high percentage of visible rot may give low mold counts. This has been confirmed in the results obtained here. However, what has not been emphasized is that low concentrations of visible rot may give high mold counts.

While Eisenberg (1952b) made no mention of it, his data showed that 2.7 percent rot produced by Alternaria gave a

TABLE V

RELATIONSHIP BETWEEN MOLD COUNTS AND PERCENTAGE ROT OF WHOLE TOMATOES

Organism	% Rot	Mold Count	Organism	% Rot	Mold Count
Alternaria	27.2	100	Fusarium	15.9	98
"	3.5	14	"	3.6	86
"	3.6	80	"	1.8	94
"	24.0	56	"	7.3	96
"	5.5	79	"	12.8	100
"	10.3	100	"	15.5	100
"	3.3	96	"	5.1	21
"	2.0	92	"	6.5	65
"	1.7	92	"	8.9	94
"	5.4	100	"	6.5	22
"	1.9	100	"	3.7	100
"	1.1	44	"	0.1	14
"	2.9	28	"	5.5	94
"	4.9	100			
"	2.1	28	Mucor	10.2	100
"	3.6	86	Oospora	30.7	100
"	0.1	12	"	7.0	82
"	3.7	48	"	17.2	98
"	11.4	42	"	3.8	72
"	3.0	28	"	1.9	100
"	1.0	69			
"	0.1	57	Penicillium	4.3	64
			"	5.0	100
Colletotrichum	17.6	100	"		
"	2.9	100	Rhizoctonia	1.8	68
"	4.8	80	"	3.6	86
"	6.9	100	"	5.1	100
"	2.8	88	"	6.1	82
"	0.9	23	"	7.1	22
"	1.7	82	"	3.0	100
"	4.2	86	"	9.4	100
"	5.7	100	"	18.5	100
"	9.1	100	"	19.4	100
"	1.8	28			
"	4.4	96	Control	0	0
"	3.3	54	"	0	2
"	1.1	64	"	0	2
"	9.5	100	"	0	8
"	2.8	46	"	0	0
"	2.7	20	"	0	2
"	4.0	28	"	0	0
"	37.1	100	"	0	2
"	2.8	63	"	0	2
"	21.2	100	"	0	0
			"	0	2
			"	0	0

a count of 53, that 3.1 percent Colletotrichum rot yielded a count of 55, and that 1.9 percent rot produced by Oospora gave a count of 55.

It can be appreciated that it would be impossible for the average mold count analyst to differentiate these types of molds in a sample of a tomato product. However, it is the type of mold which is the determining factor in the amount and type of rot present, and likewise the type of mold has an important bearing on the mold count itself as considerable variation is known to exist in the size of the filaments (Beneke, 1950). The type of mold which the Howard mold count does not and cannot determine is a more important consideration than the mere presence of infinitesimal mold fragments, which may or may not be important from the standpoint of rot.

Mold Count of Trimmed Tomatoes Compared to Mold Count of Tomatoes Which Did Not Require Trimming

It was thought of interest to compare the mold count of tomatoes which did not require trimming with those obtained by examining tomatoes which showed visible rot or mold which required trimming. Accordingly ten pound samples of tomatoes which showed many of the molds encountered elsewhere in this study were collected at a canning factory platform. From the same hampers four ten pound samples which showed no visible

rot or mold growth were carefully selected. The lots of tomatoes which showed mold or rot were carefully trimmed. All sets of tomatoes were individually comminuted and Howard mold counts and pH values were obtained on the macerates.

Also, to determine what influence, if any, the different genera might have on tomato sections remaining after the tomatoes had undergone trimming, decomposed areas were cut out from individual tomatoes and the molds therein identified. The pH values and the mold counts obtained by comminuting the individual rot and mold-free sections were compared.

The mold counts of tomatoes which did not require trimming in all but one instance were lower than those obtained from the examination of tomatoes from which all mold growth and rot had been removed (Table VI).

These findings are in agreement with observations noted by Hand et al. (1953). In this same study these investigators found that even after trimming, the mold count of U. S. No. 2 tomatoes which showed 100 percent defects other than color rose over 20 percent six times out of 12 in 1950 and three times out of 12 in 1951. By contrast U. S. No. 1 and 2 free from defects other than color, without trimming did not yield juice showing substantial mold counts during the experiment.

TABLE VI

MOLD COUNTS AND pH OF TOMATOES WHICH DID NOT REQUIRE TRIMMING AND TOMATOES WHICH REQUIRED TRIMMING

Tomatoes which did not require trimming			Tomatoes which required trimming		
Sample no.	Mold count (10 lb composite)	pH	Sample no.	Mold count (10 lb composite)	pH
1	5	4.3	1a	30	4.6
2	4	4.3	2a	41	4.4
3	8	4.3	3a	4	4.8
4	2	4.7	4a	44	4.5

TABLE VII

INFLUENCE OF MOLD GENERA ON THE pH OF TOMATOES AFTER REMOVAL OF ALL VISIBLE ROT AND MOLD GROWTH

Genus isolated from discarded area	pH of rot and mold free area	No. of tomatoes
Alternaria	4.5	50
"	4.2	4
Colletotrichum	4.6	12
"	4.3	8
Fusarium	4.5	32
Rhizoctonia	4.5	43
"	4.2	24
"	4.6	2
Phythophora	4.3	10
"	4.4	8

It will be noted also (Table VII) that the pH value of the composite samples which required trimming were generally higher than those of tomatoes which did not require trimming.

The majority of the trimmed tomatoes had a higher pH in the vicinity of 4.5. No individual genus produced a significantly higher pH than other genera. The high pH value in these trimmed tomatoes substantiated the findings obtained by the examination of the composite samples.

Influence of Factory Operations on Mold Count and pH of Tomato Juice

As laboratory experimental results frequently differ from those obtained under practical operating conditions, it was decided to investigate further the pH and mold count of comminuted tomatoes.

At a canning factory ten pound samples were taken of tomatoes as these arrived on the platform, after they had been washed and flumed and after they had been steamed, culled and trimmed. Ten pound samples of the trims and culls were collected also. The samples were comminuted through the laboratory pulper-finisher. Mold counts and pH values of the juices obtained were compared with samples of tomato juice collected immediately preceeding the evaporator.

The experiment was repeated eight times. An effort was made to sample similar lots of tomatoes as these proceeded through the factory.

The results are given in Table VIII.

After the washing, trimming and culling operations there was invariably a reduction in the Howard mold count. A comparison of mold counts obtained by sampling at the other sites did not reveal any significant information undoubtedly due to the important sampling error.

The high pH values of the trims and culls was the most outstanding finding. All of the eight samples were appreciably more alkaline than samples of incoming tomatoes. The pH value ranged from 4.8 - 5.5 in contrast to the pH range of incoming tomatoes which was 4.2 - 4.3. These samples showed 100 percent Howard mold count fields. Under the factory conditions noted trimming and culling of tomatoes made the tomato juice more acid in addition to decreasing the mold count.

It is suggested that molds may contribute to multiplication of flat-sour organisms on whole tomatoes in fields and in factories and thereby present a potential hazard to a processor.

Furthermore, if tomato juice is made from poorly culled and trimmed tomatoes and as a result shows a high pH, then this juice might be more subject to spoilage by flat-sour

TABLE VIII

INFLUENCE OF FACTORY OPERATIONS ON MOLD COUNT AND pH OF TOMATO JUICE

Source of Extract	A		B		C		D		E		F		G		H	
	pH	mold count	pH	mold count	pH	mold count	pH	mold count	pH	mold count	pH	mold count	pH	mold count	pH	mold count
Platform	4.3	51	4.3	27	4.2	31	4.2	44	4.3	4	4.2	28	4.3	65	4.3	72
After washing and fluming	4.3	71	4.3	26	4.2	17	4.2	17	4.2	16	4.2	63	4.3	51	4.3	24
After steaming, culling, and trimming	4.3	8	4.3	16	4.2	12	4.2	3	4.3	5	4.2	9	4.2	8	4.2	9
Juice line before evaporator					4.1 4.2 4.2	11 22 14	4.2 4.1	40 20	4.3	26	4.2	33	4.2 4.2	40 13	4.2	41
Trims and culls	5.2	100	4.8	100	4.6	100	4.8	100	4.5	100	5.5	100	5.3	100	5.2	100

organisms some of which have been reported to spoil only tomato juice having high pH values (Pederson and Becker, 1949; White, 1951).

Influence of Mold Growth on Flavor and pH of Tomato Juice

As a preliminary investigation of the influence of mold on the flavor of tomato juice organoleptic examinations were made of thirty-five samples of juice prepared from tomatoes which contained most genera and species of molds found in the Indiana and Ohio tomato fruits.

The entire fruits, many of which showed advanced decomposition, were comminuted. The results are given in Table IX.

Only 3 of 21 samples containing Alternaria sp. and Colletotrichum sp. produced off-flavored juice.

By contrast, four of five samples of juice containing Oospora were bitter. The sample of Rhizoctonia which showed off-flavor was nauseating. The sample of Penicillium showing flavor change was pleasant.

Subsequently tomato juice was prepared from sound whole tomatoes and dispensed in 100 ml amounts into 200 ml bottles. These were plugged with cotton and sterilized for 20 minutes at 15 pounds pressure. Duplicate sets of bottles were inoculated with spores and vegetative fragments of 27 test organisms and then incubated at 20-22°C.

TABLE IX

FLAVOR CHANGES NOTED IN TOMATO JUICE PREPARED FROM FIELD TOMATOES CONTAINING KNOWN MOLD GENERA

Organism Present	No. Samples Examined	No. Samples Showing Off Flavor	No. Samples Showing No Flavor Change
Alternaria	12	2	10
Colletotrichum	9	1	8
Fusarium	4	1	3
Oospora	5	4	1
Penicillium	2	1 improved	1
Rhizoctonia	3	1	2

The inoculated juice was examined and compared to the uninoculated controls at the end of 24, 48 and 96 hours. Four tasters participated in the examinations. The results are given in Table X.

During the initial stages of incubation some of the molds produced pleasant tastes but by the end of 96 hours bitter principles were liberated and many of the juices had changed markedly in flavor.

Tomato juice containing Penicillium showed a flavor which was preferred to that of uninoculated juice. Rhizoctonia sp. produced a very objectionable taste and odor.

No ill effects were noted after tasting of the juices.

The tomato juice had to be disturbed each time an organoleptic examination was made and some bottles were consumed to a greater degree and more subject to agitation than others, which undoubtedly influenced the growth of the molds and pH.

It was considered desirable to investigate the influence of molds on tomato juice when samples were left undisturbed, and to investigate further the influence of genus and species. The experiment was repeated employing 124 cultures. The juice was inoculated with the cultures and incubated for five days before readings were made. The results are given in Table XI.

In standing culture Rhizoctonia sp. produced the highest pH value. Fusarium, Mucor, Oospora and Alternaria yielded pH values over 5.0 in most instances.

TABLE X

ORGANOLEPTIC AND pH CHANGES IN TOMATO JUICE INOCULATED WITH MOLDS

Organism	24 hours	48 hours	96 hours	10 days	pH
Alternaria sp.	-	-	flat	6.3	4.7
"	-	-	-	4.8	5.3
"	-	-	flat	4.8	4.5
Aspergillus sp.	-	-	bittersweet	5.5	5.2
" niger	bitter	bittersweet	bittersweet	4.7	4.9
" claviformus	-	sour	sour, strong odor	4.8	6.8
Colletotrichum phomoides	-	-	sweet	5.0	5.0
" "	-	-	sweet	4.7	4.8
" "	-	-	sweet	4.8	4.9
Fusarium oxysporium	bitter	bitter	bitter pungent odor	4.8	4.9
" "	just off	bitter	bitter pungent odor	4.9	5.0
Hormodendrum sp.	sweet	sweet	sweet odor and taste	6.1	5.9
Mucor sp.	sweet	bittersweet like cider	sweet sour	4.6	4.5
"	sweet	bittersweet like cider	sweet sour	4.7	4.8
"	sweet	bittersweet like cider	sweet sour	4.6	5.2
Oospora sp.	sweet	sweet sour like sour milk	sweet sour like sour milk	4.7	4.9
"	sweet	sweet sour like sour milk	sweet sour like sour milk	4.9	5.0
"	-	sweet sour	sweet sour	4.8	4.8
Penicillium sp.	sweet	sweet	sweet	5.2	4.9
"	sweet	sweet	sweet	4.5	4.9
"	sweet	sweet	sweet	4.8	4.4
Rhizopus nigricans	sour	sweet sour	sweet sour	4.3	4.5
"	sour	sour	sour	4.9	5.0
"	sour	sour	sour	4.3	4.7
Trichoderma sp.	sweet	bittersweet	bittersweet with strong odor	4.7	4.7
"	sweet	sour	sour, unpleasant	4.7	6.8
Rhizoctonia sp.	sweet	sour unpleasant	sour unpleasant	5.3	4.9

TABLE XI

pH OF TOMATO JUICE INOCULATED WITH MOLDS AFTER FIVE DAYS INCUBATION AT ROOM TEMPERATURE (STANDING CULTURE)

Mold	No. Cultures	Min.	Max.
Alternaria sp.	2	5.2	5.7
Aspergillus clavatus	1	5.2	
Aspergillus niger	2	3.3	3.4
Aspergillus terreus	2	4.8	4.8
Aspergillus sp.	1	4.7	
Colletotrichum phomoides	7	4.4	4.9
Fusarium oxysporium lycopersici	21	4.6	6.1
Fusarium cephalosporium type	3	4.7	4.8
Mucor globosus	10	4.8	5.1
Mucor hiemalis	4	4.9	5.0
Mucor sp.	3	4.9	5.2
Oospora sp.	18	4.9	6.3
Penicillium coryophilum	11	3.5	4.6
Penicillium oxalicum	2	4.5	4.9
Penicillium purpurogenum	2	4.5	4.5
Penicillium sp.	1	4.0	4.0
Rhizoctonia sp.	11	4.1	7.1
Rhizopus nigricans	18	4.5	4.9
Rhizopus sp.	2	4.7	5.0
Stilbella bulbicola	1	4.7	
Trichoderma sp.	2	4.3	4.5
Controls	5	4.5	4.5

Discussion

From the results of this experiment it is easy to understand how canners may be confused and baffled by various mold counts obtained by their analysts and by the Government. The fact that 0.1 percent visible Alternaria rot gave a Howard mold count of 57 in one instance and in another instance the same percentage visible rot, also produced by the same mold, gave a count of 14 (Table V) demonstrates clearly the problem which may confront a processor. It is well known that it is difficult to determine the extent of visible rot with accuracy but this is the task normally assigned to housewives on trimming belts in tomato processing factories.

It is well known that Colletotrichum phomoides may readily decompose tomatoes given the proper conditions. However, it was found frequently in this study that many tomatoes showing Colletotrichum phomoides lesions could not reasonably be considered as showing rot in the commonly accepted sense of the word. Even substantially increasing personnel on a trimming line in a factory could result in failure to remove all such spots.

When all visible rot is removed from tomatoes the trimmed tomatoes themselves may show higher mold counts than tomatoes which do not require trimming. It is apparent that

unless the processor receives sound stock at his platform he is placed in an extremely precarious position regardless of expenditures for sorting, trimming, and mold count analysts. The hazards which may be presented, particularly by Colletotrichum phomoides, in incoming tomatoes are familiar to many canners.

An interesting observation was the higher pH values of tomatoes showing mold growth and the influence of the molds on the flavor of tomato juice. When it is considered how some molds have improved the flavor of other products it is not too surprising to find that some may improve tomato juice. However, no reference has been noted in the literature to any possible beneficial effects of molds in tomato juice.

Smith (1952) stated that the Government is to place emphasis on the appearance of tomatoes being put into the final product. This appears a reasonable substitute for what seems to be a cumbersome and misleading method of evaluating the quality of tomato products, the Howard mold count.

PART III

FUNGAL ENZYMES IN RELATION TO DECOMPOSITION OF TOMATO FRUITS

Introduction

It has been shown elsewhere in this study that some species of molds found in tomatoes were capable of rapidly breaking down tomato tissue and spreading throughout the fruit. By contrast, other species of molds obtained from the same sources when grown under identical conditions remained in restricted areas of the tomato and produced only minor blemishes.

It appeared desirable to investigate why the molds behaved in such a manner. This seemingly would lead to a better understanding of the importance to be assigned to the presence of various molds in tomatoes.

It was believed that a basic approach would be to consider the known factors which impart firmness to tomatoes and those secretions of fungi likely to alter the chemical composition of the fruit to the extent that decomposition would occur.

It has been known for many years that molds secrete enzymes which decompose plant tissue. However, the mode of action of these molds and the factors which influence the decomposition of tomatoes by the molds has not been fully explained.

In the past few years increasing attention has been given to pectolytic enzymes as factors in decomposition of

foods. It was believed that these enzymes might assume a more important role in the rotting of tomato fruits by molds than has been recognized previously.

Review of Literature

Role of the Pectic Substances in Plants

Kertesz and McColloch (1950) noted that their work has cast considerable doubt on the usefulness and even validity of many phases of pectin chemistry. In addition difficulties in definition have existed for many years despite the terminology being reviewed only recently by the Committee for the Revision of the Nomenclature of Pectic Substances (Kertesz et al., 1944).

General agreement appears to exist, however, that pectic substances are primarily responsible for rigidity of many plant tissues. (Kertesz, 1951).

Joslyn and Phaff (1947) presented a very comprehensive review of the newer knowledge of the chemistry of pectic substances. They pointed out that the high degree of dispersion of pectins in the matrix of cellulose of plants led many early workers to believe that pectin is actually combined with cellulose. However, it has been possible to dissolve out cellulose and still retain the form of the plant substructure.

The pectins in the cell wall and those forming intracellular substances have been considered by botanists to differ somewhat. (Branfoot, 1929). Willaman (1927) stated that it would be both unkind and fruitless to point out all the mistakes and discrepancies concerning the naming of pectic enzymes in the literature. The reader is referred to the work of Phaff and Joslyn (1947) concerning the status of these enzymes and their terminology.

Most evidence has shown that the pectic substances in the intercellular layer, or the middle lamella, are believed to occur as insoluble calcium polygalacturonates, both calcium pectinate and pectates (Joslyn and Phaff, 1947). Holden (1950a) concluded that it is the state of combination of the pectic substances rather than their inaccessibility which influences the extent of enzymic action.

Chemical and Physical Characteristics of Tomatoes

One of the first groups of workers to emphasize that good quality canned tomatoes could be obtained only by careful attention to pectic substances was Appleman and Conrad (1927). Wholeness was listed by these investigators as one of the important requirements to be attained in canned tomatoes. They pointed out that in tomatoes the two chief pectic constituents are protopectin and pectin, and that these greatly influence whether a sloppy pack of tomatoes

results. They claimed that the cells of the plants are held together by protopectin. In green tomatoes protopectin predominated but as the tomato matured a rapid transformation of protopectin into pectin by enzymatic action was reported. From the pink to the full ripe stage the ratio of pectin to protopectin doubled. When they compared firm and soft tomatoes it was shown that soft tomatoes showed a higher ratio of pectin to protopectin or conversely the less the transformation of protopectin to pectin the better the tomato. They showed that a decrease in pectic substances coincided with softening and subsequent deterioration.

Rooker (1930) first emphasized the role of pectin in tomato products other than canned tomatoes.

Kertesz (1938) claimed that canners prefer tomatoes in the full ripened stage of maturity when their pectin content is highest.

Extensive experiments (Kertesz et al., 1940; Sayre et al., 1940) concerned with the uptake of calcium to produce increased firmness of tomatoes, showed that it is the pectate substances in the tissue rather than the calcium which is responsible for firmness of tomatoes.

Kertesz et al. (1940) reported that considerable variation occurred in the calcium content of tomatoes grown in different regions. The calcium content of drained whole tomatoes ranged from 40 to 98 p.p.m. and averaged 66.2 p.p.m.

To improve the solidity of tomatoes, the calcium content had to be increased to over 100 p.p.m.

Sayre et al., (1940) showed that applications of even excessive amounts of calcium fertilizer showed practically no addition to the calcium content of tomato fruits by this method. It was concluded that dipping peeled tomatoes in a solution of calcium chloride for about 2 or 3 minutes greatly improved firmness. Calcium pectate was identified as being responsible (Loconti and Kertesz, 1941). Baker (1946) elaborated on the similar role of calcium in firming apple slices.

It has been shown that there are at least two pectic enzymes in tomatoes and possibly three. In green tomatoes, Kertesz (1938) found that pectinase was absent and that polygalacturonase (PG) was practically absent from the fruit at this stage. In this same study he reported that PG was not always detected in ripe tomatoes and then sometimes only with low activity. He used three varieties of tomatoes in his tests and concluded that pectin polygalacturonase did not appear to be a varietal characteristic. Bell (1951) found substantially similar results, and suggested that the absence of the softening enzyme in green tomatoes may account for the lack of difficulty experienced by industry in brining this commodity.

McColloch and Kertesz (1948, 1949) reported the presence in tomatoes of a heat-resistant factor possibly an enzyme.

They called this depolymerase (or DP) and concluded that the loss of pectinic substances in processed tomato products is the result of pectinesterase (or PE) which first demethylates the pectinic acids and the DP which then depolymerizes the pectic acid substrate causing a loss of the colloidal properties of the tomato product.

MacGillivray and Ford (1928) presented a valuable discussion of the contributions of the various regions, outer and inner wall, the inner locule tissue, the jelly-like pulp around the seeds and the skin of the tomato to its overall quality.

Decomposition of Plant Tissues by Molds

Most studies related to the action of fungi on plant tissues have been reported prior to 1920 (Kertesz, 1951). For many years plant pathologists have sought to determine the factors responsible for the development of parasitism in the molds.

Branfoot (1929) ably reviewed the early research. Williaman (1927) also contributed an excellent review of the relationship of fungi to the enzymatic breakdown of pectin in plants. Bate-Smith and Morris (1952) pointed out that each particular substrate under natural conditions seems to support only a limited microflora and to be almost invariably attacked by this flora. They cited how

oranges are liable to attack by Penicillium digitatum which is quite distinct from P. expansum which attacks apples.

Foster (1949) claimed the number of species of fungi known in 1938 was estimated at 89,000.

According to Brown (1948) the invasion by facultative fungi consists of three stages, (a) prepenetration stage, (b) process of penetration, and (c) post penetration stage. He stated that no fungi have been known to secrete a substance capable of dissolving the outer cuticularized surface of plants and in this same report he said that the act of penetration of plant tissue by fungi has been clearly demonstrated as being mechanical. He outlined how by the use of gelatin filters of graded hardness it had been shown that the molds, Botrytis cinerea, Penicillium glaucum and Rhizopus nigricans respectively had diminishing penetrating power. He offered this as explanation as to why Rhizopus nigricans has been found to enter only the least protected structures such as the soft fruits in contrast to B. cinerea and Rhizoctonia solani which had been found able to enter most plants by direct penetration.

Willaman et al. (1925) in studying the problem of the comparative resistance of certain plum varieties to brown rot emphasized that the more resistant varieties had a tougher skin and a firmer flesh. Accordingly, they believed that resistance was due to mechanical resistance to the entrance of the fungus.

This was investigated further by Willaman (1926). The toughness of the skin was found to increase in all varieties of plums as ripeness progressed, the change becoming more marked in the resistant varieties. Rosenbaum and Sando (1920) reported a correlation between skin toughness and resistance to Microsporium tomato (Cook).

Once inside the tissue the most outstanding feature shown has been that the cells of the tissue are disorganized in advance of the invading hyphae (Brown, 1948) and pectinase secretion by the fungus was mentioned by Brown (1948) as the most prominent agent of attack. In this report he claimed also that within limits a correlation has been detected between parasitic vigor and the capacity to secrete pectinase into the culture medium.

Many factors appear to influence the development of rot at this stage. Weurman (1952) found that there are inhibitors in pears which inhibit fungal PG but that their chemical nature and functions were not known.

Internal resistance to the growth of most fungi was mentioned by Brown (1948) to be due to such substances as acids, tannins, ethereal oils, and glucosides and he indicated that the metabolism of the organism may also cause inhibition of growth as may desiccation of the tissue itself. In some cases a gumming reaction may occur or the formation of a cork barrier also may limit growth (Brown, 1948).

Kertesz (1931) stressed that very little has been known about the discharge of enzymes secreted by molds into culture medium and pointed out that with changes in the medium, changes occur in the life cycle of the molds also, and that on account of different substrates the molds have produced different amounts of enzyme. Also he said that while there have been many observations concerning the effect of various nutrients on the formation of PG the relationship has been far from clear.

Menon (1934) also showed data which indicated that the medium greatly influenced the precise behavior of the enzymes of any particular fungus. He claimed that some of its properties are greatly influenced by absorption from the nutrient medium.

Willaman (1927) suggested that the medium appeared to exercise a quantitative and possibly a qualitative effect on the production of the enzyme. He suggested that the dissolving of the middle lamella may be one of calcium removal.

Kraght and Starr (1953) emphasized that any report that an organism does or does not produce PG should be considered in the light of the medium and conditions.

Fernando and Stevenson (1952) showed that Botrytis cinerea spores which germinated on the cut surface of fresh untreated potato tissue rarely produced any measurable attack. However, when the tissue was injected with water, definite attack

followed. They concluded that there was a factor associated with subturgid conditions in potato tissue which antagonized the action of pectinase enzyme of Bacillus cinerea but not that of Bacillus carotovorus. They believed that the close behavior of the organism and enzyme to be a strong indication that the enzyme was in reality the active agent of parasitism.

Gregg (1952) in additional experiments on the subject of injection or soaking, stated that while one might assume that soaking or injection would facilitate the diffusion of enzyme into the tissue, the water content of soaked and injected tissues was often very similar and yet these treatments gave rise to markedly different degrees of susceptibility.

Holden (1950a) reported that in leaf tissue where pectic substances occurred in combination with calcium, the enzymes pectinesterase or PE and polygalacturonase or PG did not cause complete disintegration without removal of the calcium. She stated also that tobacco leaf fibre which had taken up the maximum calcium was scarcely attacked by PG even with prolonged incubation.

Holden (1950b) believed that any differences in the action of various unfractionated fungal enzymes on fibre by Pectinol 10M and Enzyme 19AP (Rohm and Haas), Botrytis cinerea, Aspergillus aureus, Penicillium expansum and Penicillium digitatum likely was due to a difference in the relative amounts of the enzymes and not due to their presence or absence.

Reid (1950a) reported that apple extracts were readily solubilized by the action of some molds whereas in black currants the amount of enzyme resistant material was considerable. In this same report he stated that the relative amounts of the enzymes in different preparations varied considerably, depending upon the strain of the organism and the condition of growth.

The pectolytic action of organisms during decomposition of plant tissues has been a matter of concern to many workers. As early as 1929 Pitman and Cruess employed pectin solutions to investigate the hydrolytic action of microorganisms. Coles (1926) reported the digestion of pectin by many microorganisms. Joslyn (1929) and Fabian and Johnson (1938) studied pickle softening reportedly caused by pectin splitting enzymes. Couchman (1939), during an investigation of the retting of flax, indicated that pectic materials definitely were decomposed.

The liberation of enzymes by fungi is receiving increasing attention as more and more variables are recognized which cast doubt upon the results of investigations reported in the early literature (Sumner and Somers, 1947; Smythe, 1951). Elrod (1942) urged that a clear distinction be made between the fermentation of pectin and macerating action and claimed that Bergey's manual (Bergey et al., 1939) has been confusing in this regard.

Methods for examination of the pectic substances and enzymes have been receiving increasing attention (McColloch,

1952; Jermyn and Tomkins, 1950; Reid, 1951b). However, there seems to have been little added to our knowledge concerning some of the basic factors governing the liberation of enzymes by fungi in many respects such as were investigated by Harter and Weimer (1921) and Weimer and Harter (1923a, b).

Experimental and Results

Production of Pectinase (polygalacturonase) by the Molds as Determined by Liquefaction of Pectate Gel

The ability of organisms to liquefy pectate gel has been used by a number of workers (Miskeow and Fabian, 1953; Nortje and Vaughn, 1953) as a basis for separating pectolytic organisms. The Coliform Sub-Committee (1949) pointed out that the ability of an organism to liquefy a pectate gel has been commonly related to its ability to cause soft rot in plants and listed two media containing sodium pectate. The advantages of pectate gel for the demonstration of the parasitic properties of organisms were described also by Sabet (1951).

One hundred and forty-five cultures of molds obtained from tomatoes during another phase of this study were cultured on the Northern Regional Laboratory sporulation medium (Gailey et al., 1946). Spores and vegetative forms then were inoculated onto Petri plates of the pectate gel of Baier and

Manchester (1943)*. This had the following composition:

sodium hydroxide	8.0 ml
Calgon	2.5 gm
beef extract	3.0 gm
peptone	5.0 gm
sodium pectate	30 gm
water	to 1000 ml

Observations to determine liquefaction and growth characteristics were made at 3, 8 and 30 days following incubation at room temperature.

Organisms which produced no liquefaction at the end of 72 hours sometimes showed slight or pronounced liquefaction at the end of 8 or 30 days.

The presence or absence of liquefaction of the pectate gel varied according to the genus, species and strain of the organism (Table XII).

Marked liquefaction was produced by two of the two strains of Aspergillus niger tested, all of the four strains of Mucor hiemalis, all of the 16 strains of Penicillium coryophilum and all of the 19 strains of Rhizopus nigricans employed. By contrast, only two of the 17 cultures of Colletotrichum phomoides produced definite liquefaction. No pronounced liquefaction was evidenced by most strains of Mucor globosus, Alternaria solani, Oospora sp., Fusarium oxysporium, Rhizoctonia

*Preparation of this medium is described in detail (California Fruit Growers Exchange, Ontario, California, Research News Letter no. 20, reference no. 333).

TABLE XII

PECTOLYTIC AND CELLULOLYTIC ACTIVITY PRODUCED BY THE MOLDS IN TOMATO JUICE

Isolate No.	Organism	Pectate gel liquefaction[1]	Tomato Juice after 5 days		
			PG[2] mg/ml	Cellulase[3] mg/ml	pH
3	Alternaria solani	None	-	-	-
7	" "	None	-	-	-
8	" "	None	-	-	-
9	" "	None	-	-	-
10	" "	None	-	-	-
11	" "	None	-	-	-
12	" "	None	-	-	-
16	" "	None	-	-	-
17	" "	None	-	-	-
5	" sp.	None	1.1	**	5.7
19	" "	None	*	-	-
21	Aspergillus clavatus	None	*	2.8	5.2
20	" niger	+++	3.8	0.6	3.3
23	" "	+++	1.6	5.0	3.4
22	" terreus	+	0.1	**	4.8
2	" "	None	*	**	4.8
125	" sp.	None	0.1	0.1	4.7
25	Botrytis cinerea	+++	-	-	-
28	Colletotrichum phomoides	++	-	-	-
30	" "	+	*	None	4.4
31	" "	+	-	-	-

1/ Pectate gel liquefaction as detected visibly during the 30-day period: + = slight, scarcely discernible; ++ = definite liquefaction but not complete; +++ = complete liquefaction of the gel in the plate.
2/ Polygalacturonase expressed as Pectinol 10M.
3/ Cellulase expressed as Enzyme 10M.
* Less than 0.05 mg/ml polygalacturonase expressed as Pectinol 10M.
** Less than 0.1 mg/ml cellulase (Cx) expressed as Enzyme 19.
- = No determination made.

TABLE XII (Cont.)

Isolate No.	Organism	Pectate gel liquefaction	Tomato Juice after 5 days		
			PG mg/ml	Cellulase mg/ml	pH
32	Colletotrichum phomoides	None	-	-	-
33	" "	+	1.6	**	4.5
34	" "	+	-	-	-
36	" "	None	-	-	-
37	" "	None	-	-	-
38	" "	None	-	-	-
39	" "	None	-	-	-
40	" "	+	-	-	-
41	" "	+	0.1	**	4.9
42	" "	+	*	**	4.6
43	" "	+	-	-	-
44	" "	+	*	None	4.6
45	" "	+	-	-	-
46	" "	+	*	None	4.6
47	" "	None	*	**	4.6
48	Fusarium oxysporium	None	*	**	5.2
49	" "	None	0.7	**	5.1
50	" "	None	*	**	5.1
51	" "	None	*	**	4.6
52	" "	+	1.1	**	6.1
53	" "	None	0.05	**	4.9
54	" "	None	*	**	5.0
56	" "	None	*	**	4.9
58	" "	None	*	**	4.8
59	" "	None	*	**	4.6
60	" "	None	0.05	**	5.0
61	" "	+	*	**	4.7
62	" "	None	0.7	0.2	4.4
63	" "	None	*	**	4.8
64	" "	+	*	**	5.0
65	" "	+	*	0.1	4.6
66	" "	None	0.05	**	4.8
67	" "	+	*	**	4.6
172	" cephalosporium	-	*	**	4.7
1	" "	None	0.05	**	4.8
13	" "	+	0.1	**	4.8
76	Mucor globosus	None	0.7	**	4.9
77	" "	+	3.8	**	4.8
78	" "	+	3.8	**	4.8
79	" "	None	3.8	**	5.0
80	" "	++	2.5	**	4.9

TABLE XII (Cont.)

Isolate No.	Organism	Pectate gel liquefaction	Tomato Juice after 5 days		
			PG mg/ml	Cellulase mg/ml	pH
82	*Mucor globosus*	+	1.6	**	4.9
83	" "	++	6.0	**	5.1
85	" "	+	3.8	**	4.8
87	" "	+	6.0	**	5.0
89	" "	+	2.5	None	5.0
71	" *hiemalis*	+++	2.5	**	5.0
72	" "	+++	1.6	**	5.0
73	" "	+++	2.5	None	5.0
74	" "	+++	1.1	None	4.9
81	" sp.	None	*	None	4.9
84	" "	+	2.5	**	5.1
86	" "	+	2.5	**	5.2
90	*Oospora* sp.	+	0.5	**	4.9
91	" "	+	1.1	**	6.0
92	" "	+	1.1	**	5.3
93	" "	+	2.5	**	6.2
94	" "	+	1.1	**	5.8
95	" "	+	0.3	None	6.3
96	" "	+	0.5	**	5.8
97	" "	None	0.5	None	5.4
98	" "	None	1.1	0.1	6.3
99	" "	None	0.5	None	4.3
100	" "	None	1.1	None	6.0
101	" "	+	3.8	None	5.1
102	" "	None	0.2	None	4.8
103	" "	None	2.5	None	6.0
104	" "	+	1.6	**	6.1
105	" "	+	*	None	5.0
106	" "	+	*	**	5.0
107	" "	None	1.6	**	6.3
108	*Penicillium coryophilum*	+++	0.5	0.6	4.6
109	" "	+++	1.6	1.0	4.1
110	" "	+++	0.3	1.6	3.5
112	" "	+++	0.5	2.8	4.6
114	" "	+++	1.6	2.8	4.4
115	" "	+++	0.2	1.0	3.8
117	" "	+++	2.5	2.8	4.2

TABLE XII (Cont.)

Isolate No.	Organism	Pectate gel liquefaction	Tomato Juice after 5 days		
			PG mg/ml	Cellulase mg/ml	pH
121	Penicillium coryophilum	+++	1.1	1.6	4.0
122	" "	+++	0.7	2.8	4.1
123	" "	+++	0.5	1.0	3.7
124	" "	+	0.7	1.6	3.9
111	" oxalicum	++	6.0	5.0	4.5
116	" "	++	1.1	1.0	4.9
113	" purpurogenum	None	*	None	4.5
119	" "	None	*	None	4.5
120	" sp.	++	0.7	2.8	4.0
127	Rhizoctonia sp.	None	*	**	7.1
128	" "	None	*	None	4.7
130	" "	None	*	None	4.7
135	" "	None	*	None	5.0
138	" "	None	*	None	4.6
140	" "	None	*	None	4.6
141	" "	None	0.2	**	4.3
143	" "	None	1.1	0.3	4.5
144	" "	None	None	**	4.7
147	Rhizopus nigricans	+	0.1	**	4.7
148	" "	+++	0.1	**	4.7
149	" "	+	0.05	**	4.9
150	" "	+++	6.0	None	4.9
151	" "	+++	1.6	**	4.8
152	" "	+	0.2	0.6	4.5
153	" "	+	0.1	**	4.9
154	" "	++	0.05	**	4.7
155	" "	+	1.1	**	4.7
156	" "	++	0.05	**	4.7
157	" "	++	*	**	4.7
158	" "	++	*	**	4.8
159	" "	++	0.05	**	5.1
160	" "	+++	0.1	**	4.7
161	" "	+++	0.1	**	4.7
163	" "	+++	0.1	**	4.6
164	" "	+	0.2	**	4.5
166	" "	++	0.1	**	4.5

TABLE XII (Cont.)

Isolate No.	Organism	Pectate gel liquefaction	Tomato Juice after 5 days		
			PG mg/ml	Cellulase mg/ml	pH
55	Rhizopus nigricans	++	-	-	-
162	" sp.	+++	0.05	**	5.0
165	" "	None	0.5	**	4.7
167	Stilbella bulbicola	None	*	**	4.7
168	Trichoderma lignorum	None	None	**	4.5
169	" "	None	None	**	4.3

solani, Stilbella bulbicola, Trichoderma lignorum or Penicillium purpogenum.

The cultures which previously had been the most active in breaking down tomato tissue were among the most active ones in the liquefaction of the pectate gel, as for example, Aspergillus niger, isolate no. 23 and Rhizopus niger, isolate no. 150. The majority of the organisms which showed no well-marked liquefaction of the pectate gel were those which had not produced severe symptoms in tomato fruit within a short time after inoculation. This applied particularly to Alternaria sp. and Colletotrichum sp.

This test was most satisfactory in indicating the organisms which were actively pectolytic.

It was difficult to employ this test for separating organisms that only slightly liquefied pectate gel from those organisms which showed no activity. Sometimes slight liquefaction appeared under mycelial mats and this liquefaction was masked by the growth of organisms and was very difficult to discern. It was observed, too, slight liquefaction occurred only at the edge of some colonies. This was frequently noted in the Petri plates containing Colletotrichum sp.

The amount of aerial hyphae produced by the molds was not related to the behavior of these molds in liquefying pectate gel. The top plate of Figure 5 shows a Petri plate laden with hyphae produced by Mucor globosus. This organism did not

produce visible liquefaction of the gel. The small section of medium at the top of the plate became detached when the gel was pried loose to determine if liquefaction occurred under the mat. The plate at the bottom of Figure 5 shows Aspergillus sp. with almost an absence of aerial hyphae but liquefaction of the gel. When the plate was placed vertically the medium ran to the bottom of the Petri plate. Two strains of Fusarium oxysporium are shown in Figure 6. One had a considerable amount of aerial hyphae, the other had almost none. Neither culture produced liquefaction of the pectate gel.

Many differences in the colony characteristics of the various genera were apparent (Figures 7 and 8). The two strains of Alternaria solani in Figure 7 showed little resemblance to each other.

Production of Polygalacturonase and Cellulase (Cx) by the Molds in Tomato Juice as Determined by Cup-plate Assays

The present investigation was undertaken to obtain an estimate of the relative amounts of polygalacturonase and cellulase produced in tomato juice by the molds isolated.

Viscometric assays have proven very satisfactory for pectic enzymes studies (Reid, 1950a). Tests employing pectin for measuring the combined PG and PE activity of fungal extracts have been used by a number of workers (Bell et al., 1950; Bell, 1951; Phaff, 1947; Luh and Phaff, 1951; White and

Fig. 5 Pectate gel liquefaction experiments illustrated by Aspergillus sp. and Mucor globosus.

Lower plate: Aspergillus sp., medium was liquid and detached from edge of Petri plate.
Upper plate: Mucor globosus, medium was solid, not detached from edge of Petri plate.

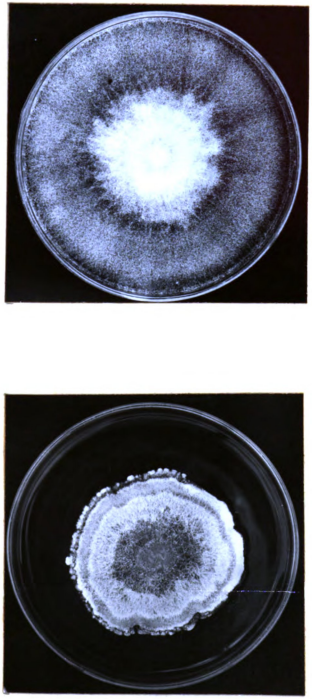


Fig. 5. Comparison of the aerial mycelia produced by two strains of Fusarium oxysporium.

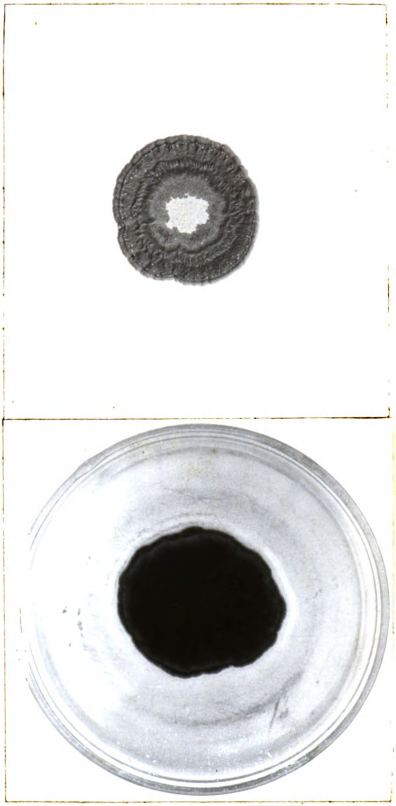


Fig.7. Differences in the growth characteristics of two strains of Alternaria solani cultured on pectate gel medium.

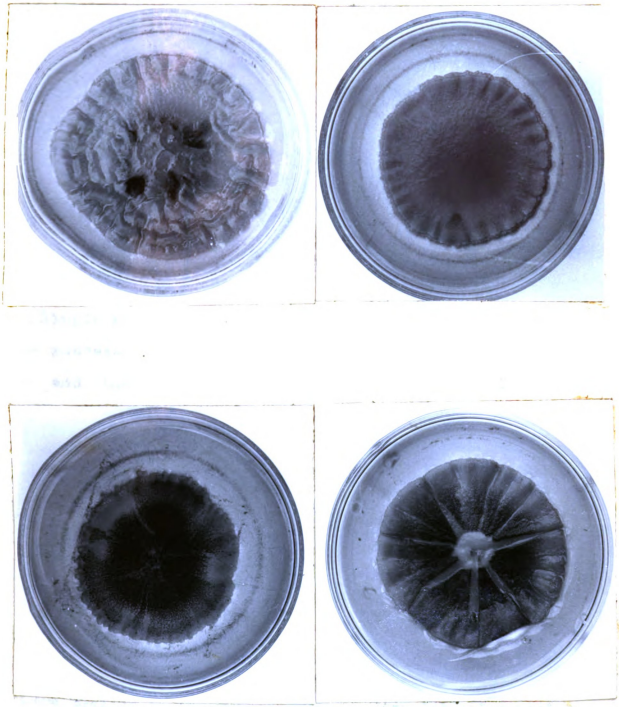


Fig.8. Differences in the growth characteristics of four strains of Colletotrichum phomoides cultured on pectate-gel medium.

Fabian, 1953; Beneke et al., 1954; Reid, 1951a). For determining PG activity alone, viscometric assays employing sodium pectate have been conducted by Reid (1951a) and Nortje and Vaughn (1953). However, some types of viscometers, particularly Ostwalds, are cumbersome to use and errors may result if minute particles are present in the preparations (Kertesz and Loconti, 1944). A criticism of methods was made by Pandhi (1953).

The method selected was essentially that of Reid (1950b) and Dingle et al. (1953), a cup-plate procedure which has been used successfully for screening preparations for PG, cellulase (Cx) and other enzymes (Dingle and Solomons, 1951, 1952). It was not the intention of this study to measure small amounts of PG which might be produced by some of the molds or that PG which might be present normally in the tissue of some tomato fruits. (Kertesz, 1938; MacDonnell et al., 1945; Bell, 1951).

Briefly, the method selected consisted of placing minute amounts of the preparations being tested into cups which were cut in the medium. After incubation with controls the plates were flooded with a reagent, readings made and the amount of enzyme activity present determined from a standard curve. As the details of the method have not been readily available they are given here.

For the PG assay, the medium consisted of 5 grams ammonium oxalate (to remove any calcium present), 10 grams sodium pectate,* 0.1 gram salicylanilide (to prevent mold growth),

*Sodium polypectate, obtained from Sunkist Growers, Ontario, California.

20 grams agar, brought to 1 liter with potassium dihydrogen phosphate buffer, pH 4.5. The reagent was 5N hydrochloric acid and the standard was Pectinol 10.** For the cellulase assay the medium consisted of 10 grams sodium carboxylmethylcellulose, 0.1 gram salicylanilide, 20 grams agar, brought to 1 liter with Walpole's acetate buffer pH 4.5 (Hawk et al., 1951). The reagent was a 10 percent solution of copper acetate.

Pectinol 10M was employed as a standard for measuring both PG and cellulase by Dingle and Solomons (1952). Unlike PG, the cellulase content of Pectinol 10M has not been standardized from batch to batch (Labbee, 1954), whereas Enzyme 19** has been standardized for cellulase content and therefore was employed in this study. Both standards were kept at 5°C under silica gel.

Specially constructed stainless steel and glass frames having an internal surface area of 6 1/2" x 12 1/2" were made to hold the media. To conduct an assay the glass plates were wiped with ethanol and warmed by placing in an incubator at 60°C. Plates were poured by adding a standard amount of the medium at 60°C to provide a 4 mm layer. A spirit level was used to check even distribution of the medium. The medium was poured on part of the plate with the minimum of tilting and bubbles were broken with a hot needle. When the agar was set (generally 30 minutes) a paper template was placed under the

**Pectinol 10M obtained from Rohm and Haas Company, Philadelphia, Pa. Enzyme 19 obtained from the same source.

glass and cups 8 mm in diameter were made in the agar with a sharp cork borer. Those agar discs were removed by a diamond shaped needle. The center of the holes were approximately 4 cm apart.

Accurate amounts of each preparation under test were added to duplicate cups employing a rubber bulb attached to a piece cut from the end of a capillary pipette, the glass of which had been drawn out to provide delivery of 0.06 ml of the preparation under test. Between tests the filler was rinsed with pH 7.0 buffer and distilled water. Duplicate cups containing four logarithmic concentrations of the control were included on each plate. These control concentrations were prepared with tomato juice immediately preceeding an assay.

The plates were covered with aluminum foil to prevent evaporation and placed in a 37°C incubator. After 18 hours the plates were removed, flooded with reagent and the readings made.

Brownlee et al. (1948) recommended that in testing preparations the cup-plate zones be magnified. A Delineascope apparatus provided with an accessory stage rack to hold the plate proved very satisfactory (Figure 9). This was arranged so that segments of plates were magnified six times and projected onto a grid. In the PG assay, the diameter of the sharp, inner area of hydrolysis was measured (Dingle et al., 1953). In the cellulase assay the outer zone diameter was measured directly from the plates with calipers.

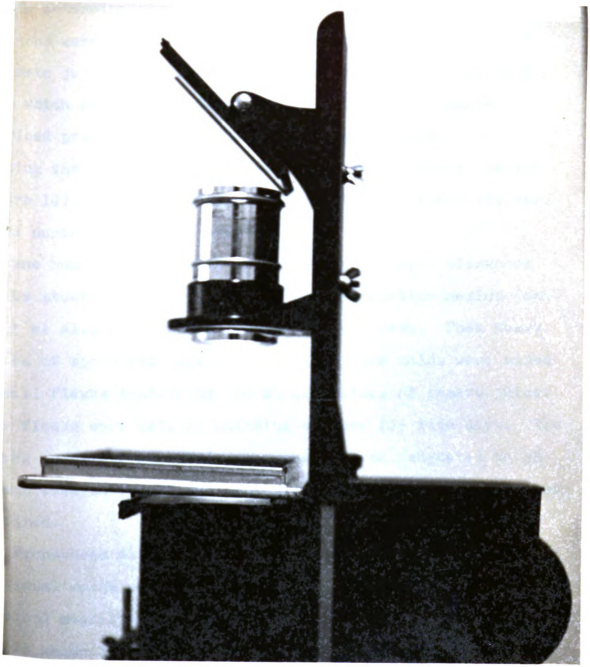


Fig. 9. . Modified Delineascope apparatus employed to magnify and project in the estimation of poly galacturonase activity in tomato extracts and cultures.

To determine the sensitivity of the tests logarithmic dilutions were made of a 4 percent solution of Pectinol 10M in tomato juice which was placed in quadruplicate cups in a plate which was then incubated and treated in the manner described previously. Standard curves were prepared by plotting the zone diameters against the enzyme concentration (Figure 10). Readings were made directly from these standard curves during subsequent sample tests.

One hundred and twenty of the molds employed elsewhere in this study were transferred to the sporulation medium (see Gailey et al., 1946) and incubated for one week. Then heavy inocula of spore and vegetative cells of the molds were added to small flasks containing 100 ml quantities of tomato juice. These flasks were left in standing culture for five days. The liquids under the mycelial mats were removed, adjusted to pH 4.5 and assayed for PG and cellulase (Cx) using the method just described.

Pronounced differences in the production of PG by the individual molds were apparent (Figure 11). A photograph of a typical magnified section which was projected onto the grid is shown in Figure 12. The results are given in Table XII (page 79). As was to be expected from the results obtained earlier in this investigation, most of the preparations showed an increase in pH value due to the presence of molds.

With few exceptions, little or no PG activity was shown in the tomato juice samples containing Alternaria solani,

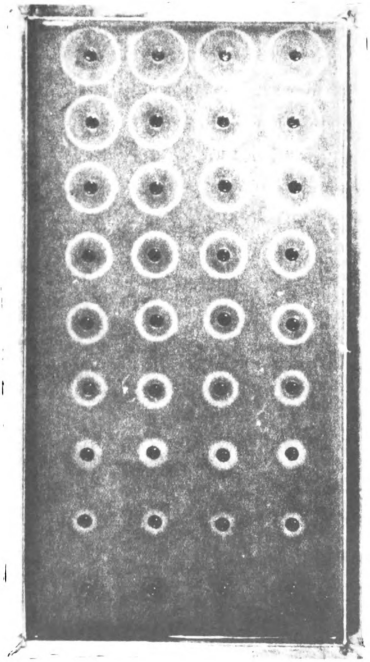


Fig. 1 . Decreasing* logarithmic concentrations of mold polygalacturonase as shown by the cup-plate assay (Pectinol 10M).
*top to bottom

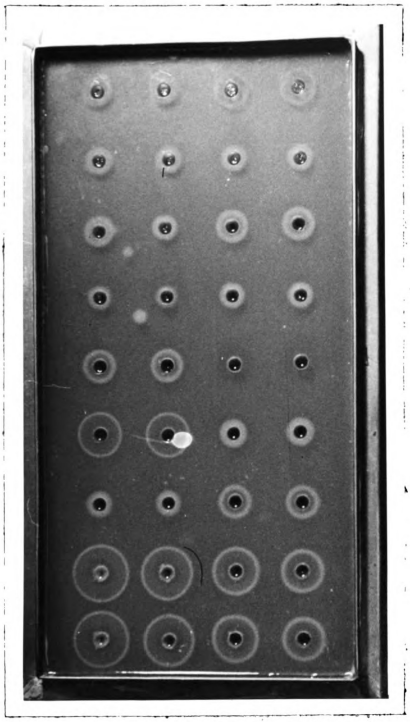


Fig. 11. Variation in polygalacturonase potency produced by 14 different molds in tomato juice.
Duplicate assays of each extract were placed horizontally.
The bottom two rows were controls of standard rectinol 10[illegible] arranged vertically.

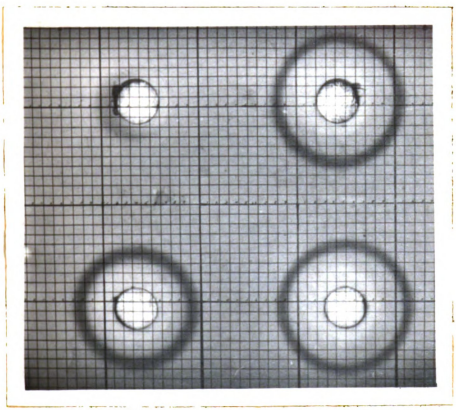


Fig. 12. Cup-plate zones magnified on grid showing measurable polygalacturonase activity in three tomato extracts. No measurable activity in one sample. X 1/4.

Colletotrichum phomoides, Aspergillus clavatus, Aspergillus terreus, Fusarium oxysporium, Fusarium cephalosporium, Rhizoctonia sp., Stilbella bulbicola and Trichoderma lignorum.

This was in contrast to considerable PG activity which was shown in samples inoculated with Mucor sp., Oospora sp. and Rhizopus sp., the molds which had been the most active in attacking tomatoes under experimental conditions.

The test for cellulase also proved satisfactory and the differences in the production of this enzyme by the various molds were readily discernible (Figure 13).

Of the molds tested, all cultures of Penicillium coryophilum and Penicillium oxalicum were unique in producing cellulase consistently and at a high level.

Strains of Penicillium coryophilum produced this enzyme in appreciably greater quantities than any of the other molds studied.

Mucor globosus and Mucor hiemalis and Mucor sp. were shown to produce a high titre of polygalacturonase in tomato juice but a low cellulase titre. This was noted also in respect to Oospora sp. These same strains of Mucor globosus, Mucor sp. and Oospora sp. had shown no liquefaction of pectate gel.

Most of the organisms produced cellulase but only in small amounts. (Table XII). There appeared to be no correlation between cellulase production and PG production by the molds.

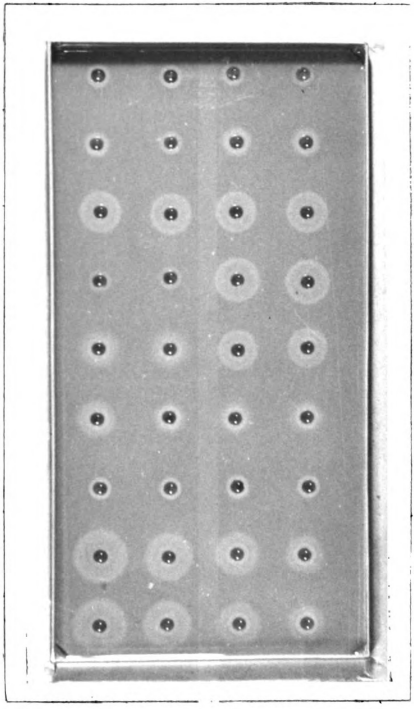


Fig. 13. Variation in cellulase (Cx) potency produced by 14 different molds in tomato juice.
Duplicate assays of each extract were placed horizontally
The two bottom rows were controls of standard Enzyme 19 arranged vertically.

Polygalacturonase Concentrations in Partially and Wholly Decomposed Areas Trimmed from Tomatoes

Tomatoes which showed macroscopic evidence of mold growth were collected from fields in Indiana and Ohio as outlined elsewhere. At the site of each mold invasion a 10 gram quantity of the visibly affected area was cut out with a scalpel. To this was added a small amount of two percent saline solution and the mixture was ground in a Waring blender. The pH was determined with a Beckman model G pH meter. One mixture was adjusted to pH 6.0 with NaOH, the other was left at the pH level noted. Each preparation was filtered through cheesecloth and brought to a volume of 50 ml with saline, poured into a test tube, layered with toluene and held at -10°C until examined. This method was essentially the same as that given by Bell *et al.* (1951). The samples were examined for PG activity by the cup-plate method described previously.

Results are given in Table XIII. Only 11 of the 208 samples of wholly or partially decomposed tissue trimmed from tomatoes showed concentrations of PG in excess of 0.05 mg/ml compared with an arbitrary strength of Pectinol 10M. Greater activity was noted only in samples from which *Mucor*, *Oospora* and *Rhizopus* had been isolated.

No significant differences were found in the PG content of samples which had not been adjusted to pH 6.0 as compared to adjusted samples. Generally, the extracts which showed high concentrations of PG also showed low pH values.

TABLE XIII

POLYGALACTURONASE CONCENTRATION AND pH OF PARTIALLY AND WHOLLY DECOMPOSED AREAS TRIMMED FROM TOMATOES

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml [1]	Organism Isolated
9a	soft, brown	6.4	None	Rhizoctonia solani
9c	soft, overripe, green and black growth	5.2	None	Alternaria, Cladosporium, Rhizopus
9d	soft, yellow and green rings	6.1	None	Rhizoctonia
9e	firm, black growth in splits	4.7	None	Alternaria
9f	soft, sunken spots, brown	4.9	None	Colletotrichum phomoides
9g	soft, greenish yellow	6.4	None	Rhizoctonia and bacteria
9h	soft, white aerial growth	8.4	None	Fusarium
9i	soft, greenish	5.2	None	Rhizoctonia, some Mucor
9j	firm, sunken, shriveiled, black	5.7	*	Mucor, Rhizopus Alternaria and Fusarium on surface
10a	soft, pinkish white hyphae	7.0	None	Fusarium
10b	soft, pinkish white hyphae	8.4	None	Fusarium
10c	soft, sunken spots, brown	4.5	None	Colletotrichum
10d	soft	7.7	None	Fusarium with with bacteria
10e	soft spots, sunken, twelve spots	6.7	None	Colletotrichum
10f	soft spots, sunken, dark, six spots	5.3	*	Colletotrichum
10g	firm, sunken, black spots, four	5.7	None	Colletotrichum
10h	soft area, 1" diameter	6.5	None	Fusarium
10i	soft spots 1" diameter on surface	5.4	None	Colletotrichum on surface, some Fusarium
10j	soft spots, sunken, concentric rings	4.9	*	Colletotrichum
11a	soft area 2" diameter	7.0	None	Rhizoctonia
11b	soft spot 1" diameter, dark	5.3	None	Alternaria
11c	soft spot 1/4" diameter	4.5	*	Penicillium
11d	soft, decomposed	4.4	*	Mucor
11e	soft, black spot	7.6	None	Fusarium, Alternaria
11f	soft, overripe, 1/2" crack	4.8	None	Rhizopus, some Oospora, Mucor on surface
11g	soft	7.6	None	Fusarium

[1] Polygalacturonase expressed as Pectinol 10M.
* Less than 0.05 mg/ml polygalacturonase expressed as Pectinol 10M.

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml	Organism Isolated
11h	3 spots, 3/4" diameter	4.9	*	Rhizopus, Oospora, yeast
11i	insect injury entirely soft	6.4	*	Penicillium, Oospora, Alternaria
11j	soft spot 3/4" diameter, black	4.8	*	Alternaria, Rhizoctonia
12a	soft area entirely	5.1	*	Fusarium, yeast
12b	dried, black surface on soft tomato	7.8	None	Colletotrichum
12c	very soft, a split	5.5	*	Oospora
12d	black spot 1-1/2" diameter	6.1	None	Alternaria
12e	soft, water-soaked areas	6.4	None	Rhizoctonia
12f	soft, dry	7.6	None	Fusarium, Oospora on surface
12g	soft, greenish yellow 1/2" diam.	6.4	*	Rhizoctonia
12h	soft spot, 1" diameter broken skin.	5.0	*	Oospora
12i	soft, decomposed	7.2	None	Rhizoctonia
12j	3 spots, 1/2" diameter	4.5	*	Colletotrichum
13a	soft, water soaked	4.5	*	Rhizoctonia
13b	firm, black spots, green edges	3.5	*	Rhizoctonia
13c	soft, brownish rings	5.0	0.05	Rhizoctonia
13d	soft, brown spot, 2" diameter	7.1	None	Rhizoctonia
13e	soft, greenish	7.5	None	Rhizoctonia, yeast
13f	soft, skin broken	6.1	*	Mucor, Oospora
13g	soft	6.6	*	Rhizoctonia
13h	soft, grayish	6.0	None	Rhizoctonia
13i	soft, greenish	7.0	None	Rhizoctonia, yeast
13j	spots 1/4-1/2" diameter	4.7	None	Colletotrichum
14a	soft, brownish spot 2" diameter	7.8	None	Fusarium
14b	sunken, tough tissue	6.8	None	Fusarium, Alternaria, Mucor
14c	dark, sunken, shallow spots	6.8	None	Fusarium, some Rhizopus on surface
14d	firm, black, sunken spot	4.3	None	Alternaria, Fusarium
14e	soft, 1/2" dark, watery	4.6	*	Fusarium
14f	soft, black spot	5.4	*	Mucor, bacteria
14g	watery spot, dark with green centers	5.4	None	Fusarium, Mucor
14i	shallow, sunken spot, 3/4" diam.	4.7	None	Mycelia
14j	shallow, brownish black spots	4.5	None	Mycelia
15a	shallow, black cracks	4.5	None	Fusarium. Some Alternaria on surface
15b	shallow, 1" diameter spot, dry	6.2	None	Alternaria, Fusarium

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-Out Area	pH	PG mg/ml	Organism Isolated
15c	shallow, 1-1/2" sunken spot	4.4	*	Rhizoctonia. Some Oospora on surface
15d	dry, brownish black 3/4" diameter, shallow	4.6	None	Alternaria
15e	soft, brown split	6.4	None	Rhizoctonia
15f	soft, brownish, watery	5.5	None	Rhizoctonia. Some Alternaria on surface.
15g	soft, brownish	5.5	None	Rhizoctonia. Some Stilbela on surface
15h	soft, dark brown rot	4.0	*	Rhizoctonia
15i	dark, brownish black rot	5.3	*	Oospora. Some Mucor, Stilbela on surface
15j	black, watery rot, foul odor	3.5	0.05	Mucor, Oospora
16a	tough, leathery, black growth from 1" diameter spot	4.8	None	Rhizoctonia
16b	sunken spot, completely rotten	5.0	*	Rhizoctonia, Alternaria
16c	shallow, watery, brown spot 1" diameter, insect hole	4.7	0.05	Rhizoctonia, Oospora
16d	all black soft rot, watery	5.2	*	Mucor, Oospora
16e	all soft, from wide crack showing slimy growth, color normal	4.3	0.2	Rhizoctonia, Oospora
16f	1-1/2" soft brown area, showing concentric rings, split	4.8	*	Oospora
16g	superficial spots, light brown	4.3	None	Mycelia
16h	superficial black, sunken 3/4" diameter	4.8	None	Colletotrichum
16i	shallow, 1" diameter black spot pink surface mold	6.1	None	Fusarium
16j	completely rotten area from fruit eaten by hoppers, covered with mold	5.1	0.05	Oospora, Alternaria
17a	all black rot	6.7	*	Rhizoctonia
17b	all soft rot from 2" area	6.4	None	Rhizoctonia
17c	very little rot, soft from insect injury	4.6	None	Alternaria, Oospora
17d	thin layer of rot in crevice	4.5	0.1	Mucor
17e	all dark brown soft rot	5.0	None	Fusarium, bacteria
17f	small proportion of rot, soft accompanying insect injury	4.4	*	Mucor, Oospora
17g	all granular rot, from cracks showing orange mold and shrivelling	7.5	None	Fusarium
17f	soft rot from insect injury	4.4	*	Mucor, Oospora

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml	Organism Isolated
17g	all granular rot, color normal	7.5	None	Fusarium
17h	only a film of visible rot, balance slightly soft from cracks	6.8	None	Fusarium
17i	all green rot, from 1" round soft spot covered with yellow mold	7.6	None	Fusarium
17j	thin scab from cracks on surface	4.5	None	Alternaria
17k	all dark rot from a 3" area containing a crack	4.5	None	Rhizoctonia, Oospora
17l	thin scab from 3/4" soft rot spot	5.2	None	Colletotrichum
17m	all soft rot from wide crack across 1/2 of fruit, creamy growth	4.6	*	Oospora
17n	thin scab from an area eaten away by insects	4.0	*	Penicillium, Alternaria
17o	thin scab from a dry sunken spot 1" diameter	4.9	None	Alternaria
17p	very little rot from a shallow soft spot 1-1/2" diameter	4.7	*	Fusarium
17q	very little rot from a 1" diameter insect injury	4.3	0.1	Oospora, some Rhizopus on surface
17r	all soft rot, color normal from 1/2 of soft tomato	7.4	None	Colletotrichum, Rhizopus
17s	tough, soft rot from cracked fruit	7.2	None	Fusarium, Alternaria
17t	pulpy, decayed tissue from black cracks	7.1	None	
18a	all soft rot, red	4.8	None	Fusarium, Alternaria
18b	all soft granular rot	7.2	None	Fusarium, Alternaria
18c	all soft, black rot	7.2	None	Colletotrichum
18d	all soft, dark rot from fruit showing crack	5.9	None	Fusarium, Rhizoctonia
18e	soft, mushy tissue from fruit with cracks	3.9	0.3	Rhizopus
18f	soft tissue from a firm brown area	4.9	None	Colletotrichum
18g	soft spot 3" diameter	5.3	None	Fusarium, Colletotrichum
18h	soft, dark rot from a dry, black spot with surrounding soft area	6.2	None	Alternaria, Colletotrichum
18i	yellow, small brown spot	4.4	None	Colletotrichum, Alternaria

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml	Organism Isolated
18j	dark, tough rot	4.4	None	Alternaria, Colletotrichum
19a	dark, soft rot	5.8	None	Rhizoctonia
19b	all dark soft rot	7.2	*	Rhizoctonia
19c	all soft rot, greenish	7.0	0.05	Rhizoctonia
19d	all soft rot, orangish brown	7.6	None	Fusarium
19e	all dark dry rot	4.4	*	Fusarium. On surface Alternaria
19f	1/2 dark rot, 1/2 normal tissue from cracks showing black growth	4.4	None	Alternaria, Fusarium
19g	all firm brown rot	6.9	*	Fusarium
19h	all soft brown tissue	8.4	None	Fusarium
19i	1/2 hard black rot, 1/2 soft brown rot	6.6	None	Fusarium
19j	brownish black rot	8.0	None	Fusarium, Oospora
20a	thin surface scab	4.0	None	Aspergillus, Rhizoctonia
20b	soft rot, dark	5.7	None	Rhizoctonia. Some Fusarium on surface
20c	all rot, soft	6.9	None	Mucor, Rhizoctonia
20d	soft tissue	4.2	None	Yeast. Bacteria on surface
20e	minute black spot	4.3	None	Alternaria
20f	dark brown mottled surface spot	4.3	None	Bacteria. Fusarium on surface
20g	all brown rot	6.9	None	Rhizoctonia
20h	slightly soft tissue from cracks	3.9	0.05	Rhizopus
20i	small amount of black rot from cracks	4.8	None	Alternaria
20j	small amount of black tissue	4.1	None	Alternaria, Fusarium
20k	1/3 of sample rotten	4.2	None	Alternaria, Fusarium
21a	brown rot, foul odor	6.1	None	Oospora, Mucor
21b	heavy black rot	6.7	None	Fusarium
21c	all white rot	7.3	None	Fusarium, bacteria
21d	soft, foul odor	6.1	None	Mucor, Fusarium
21e	white rot in insect injury	5.0	0.05	Oospora, Mucor
21f	small amount of black tissue	6.5	None	Alternaria, Fusarium
21g	small amount of black tissue	4.7	None	Fusarium
21i	dry, hard rot	7.0	*	Fusarium
21j	dark rot	6.5	None	Fusarium, yeast

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml	Organism Isolated
22a	soft, slightly brown color	5.0	None	Rhizopus, Mucor
22b	3/4 rotten section	6.6	None	Alternaria
22c	dark, pulpy black rot	7.7	0.05	Alternaria
22d	1/2 soft red tissue	5.5	None	Alternaria, bacteria On surface some Rhizoctonia
22e	1/2 brown rot	4.4	None	Colletotrichum
22f	all brown, firm rot	6.0	None	Rhizoctonia
22g	2/3 dry, black rot	4.8	None	Alternaria
22h	all dark brown, firm rot	5.2	*	Rhizoctonia
22i	3/4 soft, brown rot	5.	None	Rhizoctonia
22j	all soft, red rot	4.2	0.3	Rhizopus, yeast
23a	black, hard rot	5.3	None	Alternaria
23b	3/4 soft, red rot	4.8	0.05	Mucor
23c	all firm, black rot	7.2	None	Alternaria
23d	all soft, red rot	5.6	1.1	Mucor
23e	1/2 dry black brown rot	5.1	None	Alternaria
23f	all soft, red rot	5.0	None	
23g	all soft red rot	4.8	*	Rhizoctonia
23h	all soft red rot	4.8	*	Rhizoctonia
23i	all soft red rot	4.7	0.3	Mucor
23j	small amount of black tissue	4.6	None	Alternaria
24a	1/3 black tissue	4.8	None	Alternaria
24b	1/3 soft tissue, some small sunken spots	4.7	None	Colletotrichum
24c	1/2 brown rot	5.0	*	Colletotrichum
24d	all soft red tissue	4.1	*	Colletotrichum, bacteria
24e	all soft brown tissue	6.6	None	Alternaria
24f	1/2 dry black tissue	5.5	0.05	Fusarium. Some Oospora on surface
24g	all dry, black rot	5.7	None	Rhizoctonia
24h	all soft red rot	5.6	*	Oospora, Rhizopus
24i	all soft, red tissue	6.9	None	Colletotrichum
24j	1/3 dry, black rot	5.2	None	Rhizopus
25a				
25b	all dry, black rot	5.6	*	Alternaria
25c	1/2 dry, black rot	4.9	None	Alternaria
25d	1/2 soft red tissue, 1/2 black tissue	6.9	None	
25e	greenish orange rot	4.9	-	
	all soft red rot	5.0	None	Colletotrichum, yeast

TABLE XIII (Cont.)

Sample No.	Characteristics of Cut-out Area	pH	PG mg/ml	Organism Isolated
25f	3/4 soft brown tissue	4.8	None	Rhizoctonia
25g	all soft brown rot	4.8	*	Rhizoctonia
25h	1/2 soft red rot	5.0	None	Colletotrichum. Surface Hormodendrum, Alternaria
25i	all firm black rot	6.6	*	Alternaria
25j	all soft, brown rot	5.3	None	Mucor, Rhizoctonia
26a	all soft, dry rot	7.5	None	Alternaria
26b	soft tissue	6.1	None	Fusarium
26c	soft spot	4.8	*	Rhizoctonia
26d	soft, tissue	5.3	None	Fusarium
26e	firm, black tissue	5.4	None	Alternaria
26f	all soft brownish red rot	5.1	None	Rhizoctonia
26g	soft, brownish red tissue	5.4	None	Rhizoctonia
26h	soft, brownish red tissue	5.3	None	Rhizoctonia
26i	soft, dark red tissue with black spots	6.5	None	Alternaria, bacteria
26j	firm, black tissue	7.4	None	Alternaria
27a	soft, mushy tissue, red	4.5	None	Oospora
27b	soft, grayish black tissue	4.3	*	Oospora
27c	soft, light brown tissue, 9 shallow spots	6.3	None	Colletotrichum
27d	soft, light brown tissue from a fumed area of spots	5.0	None	Colletotrichum
27e	soft tissue	5.4	*	Mycelia
27f	soft tissue	4.3	*	Rhizopus
27g	all black tissue	6.0	None	Alternaria
27h	all soft rot	4.8	0.3	Rhizopus. Mucor on surface
27i	water soaked tissue	5.2	None	Colletotrichum. Oospora
27j	all dark red rot, soft	5.3	*	Rhizoctonia
28a				
28b	small amount soft tissue	5.2	None	Phythophthora
28c	small amount soft tissue	5.2	None	Phythophthora
28d	soft tissue from 2" spot	5.3	None	Phythophthora
28e	soft tissue from small spot	5.2	None	Phythophthora
28f	all soft tissue	6.0	None	Phythophthora
28g	all soft tissue	6.0	None	Phythophthora
28h	all soft tissue, green fruit	6.1	None	Phythophthora
	all soft tissue, green fruit	5.4	None	Phythophthora

Discussion

In Table XII it will be seen that the molds which did not liquefy pectate gel generally did not produce a high titre of PG in tomato juice and that this was below 0.05 mg/ml. However, in some instances when no liquefaction of pectate gel was apparent as, for example, by Mucor globosus and Oospora sp. the concentration of PG produced by such molds in tomato juice was substantial. A comparison of the pectolytic activity as shown on both media would not be expected to show identical results.

The lack of agreement between the pectate gel liquefaction test and the cup-plate assay could be attributed to many factors. The two media were not identical. Even minor differences in the medium on which fungi are grown have been reported to influence enzyme production by fungi (Kertesz, 1931; Menon, 1934).

Also, changes in the pH of the media as the molds developed may have influenced the concentration of the enzymes present (Fernando, 1937). It has been suggested that proteolytic enzymes produced by fungi may destroy PG (Luh and Phaff, 1951).

Furthermore, one should not overlook the appreciable time factor involved in the testing of the organisms and in the sample storage. In the pectate gel tests the organisms were in contact with the medium for a period of thirty days, while in the tomato juice there was a period of five days.

The density of the sowing of the spores and also the number of spores which developed on the media (Brown, 1917) also might have had an influence on the concentration of enzyme produced.

Results from the cup-plate assay technique for the estimation of PG has been shown to coincide with a loss of colloidal properties and to viscosity measurements (Dingle et al., 1953). The test for liquefaction of pectate gel is based on a loss of colloidal properties (Coliform Sub-committee, 1949).

Only one reference was found concerning the application of the pectate gel medium in determining the pectolytic properties of molds and this made mention of only two mold cultures (Misekow and Fabian, 1953).

The present study shows that the liquefaction of pectate gel by some molds may require a considerable period of time and that some liquefaction may be masked by mycelia.

This study indicates that the molds isolated from tomato fruits vary in their ability to produce pectolytic and cellulolytic enzymes.

The cup-plate assay while more intricate than the pectate gel test was very satisfactory in showing these variations and was free from the errors associated with viscometric tests.

Representatives of the genera, Mucor, Oospora and Rhizopus when inoculated experimentally into tomato juice produced an appreciably higher concentration of PG than did all of the

other organisms tested. It is interesting to note that of the many areas trimmed from partially and decomposed tomatoes, only those tomatoes from which Mucor, Oospora and Rhizopus had been isolated also contained a high PG concentration.

A loss in pectic constituents in tomatoes has been reported to coincide with softening and deterioration of this fruit (Le Crone and Haber, 1933). Dryden et al. (1952) on the basis of a study of apple pumice similarly believed that it was the decomposition of pectin which produced rot in apples. Kertesz and Loconti (1944) similarly reported that commercial pectinase caused a decrease in the gross viscosity of tomato juice solids. Obviously the tests employed in the present study were effective in indicating the pectolytic activity of the molds and the production of PG by these molds correlated with the severity of their attack of tomato fruits under experimental conditions. Tests for galacturonic acid as an indicator of decomposition have not been successful (Almendinger et al., 1954). A possible explanation for this may be the utilization of galacturonic acid by the molds themselves (Kraght and Starr, 1953).

The results of Dingle and Solomons (1952) showed that Cx was produced in appreciable quantities by a high percentage of the organisms tested. The production of great measurable cellulase activity in the present study appears to have been dependent on the genera and species (Table XII). A discussion of the cellulase production of microorganisms will not be

entered into. This is well covered in the literature (Fuller and Norman, 1945; Saunders et al., 1948; Elwyn et al., 1950; Reese et al., 1950).

It would be very desirable to investigate pure enzymes preparations many of which have been made available only during recent years (Holden, 1950b; Scheffer and Walker, 1953).

GENERAL DISCUSSION

One could not have anticipated the many types of molds which were found in Indiana and Ohio tomatoes or the pronounced variations shown by these molds in their ability to produce physical and chemical changes in tomato fruits.

It appears that species of Fusarium, Mucor, Oospora and Rhizopus may be found more commonly in field tomatoes than generally has been noted in the literature. In this study other molds such as species of Trichoderma, Mycelia sterilia and Hormodendrum were found in addition to those which have been associated generally with tomatoes. It is likely that many other genera occasionally may be found on tomatoes and, while they may contribute to Howard mold counts, they may be responsible for only slight or no damage to tomato fruit.

It would appear that in accordance with the many variables associated with the pathogenicity of organisms attacking the human body, there are many factors which influence the parasitic behavior of molds affecting tomatoes. It is apparent that one should not regard all molds as being of equal significance in producing rot.

Two relatively common molds present during the growing season on field tomatoes, Alternaria solani and Colletotrichum phomoides frequently were responsible for only minor blemishes

in the tomatoes. By contrast, pronounced degradation was observed in tomatoes from which species of Mucor, Rhizopus, Oospora, Fusarium and some other molds were isolated. Substantially similar results were found upon reinoculation of the molds into tomatoes.

It was interesting to observe that when Rhizopus nigricans, the common bread mold was introduced into tomatoes a rupturing of tissue resulted. The cause of cracks in tomatoes has been reportedly obscure (Howard, 1937). It may be that the rate at which molds liberate enzymes into tomato tissue is responsible, as well as weather conditions, for some of the cracks which are found in tomatoes. Correlation of the study shows that R. nigricans produced a high concentration of the enzyme PG in tomato juice and liquefied pectate gel. It appeared representative of molds which behaved in substantially the same manner as species of Mucor and Oospora.

By contrast, cultures of other molds, particularly species and strains of the genera Alternaria and Colletotrichum generally did not cause severe damage to tomato tissue in a short period of time. These same molds produced little or no detectable PG in any of the media tested. It seems that PG produced by most tomato molds is the principal agent by means of which molds damage tomato fruits rapidly. It is contended that it is the relative amount of enzymes produced by these molds, not their presence or absence which is of prime importance. This is in agreement with the views expressed by Holden (1950b).

The instance in which the Howard mold count showed excess of 50 percent positive fields when only 0.1 percent visible rot was present emphasizes how a low percent of visible rot may mean Government seizure of a pack while at other times this amount of visible rot would yield an acceptable Howard mold count. That high mold counts can be produced when such minute amounts of visible rot are present may explain why some canners have difficulty in complying with Government mold count standards. This would seem to apply particularly when only minor blemishes such as might be caused by Alternaria and Colletotrichum appear on tomato fruit.

One should not ignore the fact that the size of mold filaments also would be expected to have an appreciable influence on mold counts (Beneke, 1950). This might explain even more fully how high mold counts can be obtained when the amount of visible mold damage is slight. Similarly, the fragility of the mold fragments logically would assume importance. Darling had similar views (1922).

It was noted that the amount of visible mycelium produced by a mold was not related to the amount of pectolytic enzymes produced by the mold. This substantiated the results reported elsewhere (White and Fabian, 1953; Fernando, 1937).

Mold counts made on tomatoes from which all signs of visible rot had been removed were almost consistently higher than counts obtained by comminuting tomatoes which did not require any trimming. This confirms similar findings noted by Hand et al. (1953).

The question arises as to what a processor can do if confronted by a poor growing season and the necessity of extensive trimming of the tomatoes. It is obvious that he is placed in an unfortunate financial position as a result of extensive labor costs and, in addition, may be confronted with a higher mold count than that of a competitor in another section of the country where tomatoes are produced freer from blemishes and areas which require trimming.

Tomatoes from which rot had been removed were less acid than tomatoes which did not require trimming. Analyses showed that the pH value of trims and culls ranged from 4.5 - 5.8 as compared to that of 4.3 of the incoming tomatoes. It is not unlikely that some mold growth present in tomatoes may make them less acid and thereby improve the flavor. At the same time it has been suggested that the more alkaline conditions which prevail could increase the possibility of flat-sour spoilage.

Some processors have contended that tomatoes may show substantial mold growth and yield a better flavored juice than tomatoes free from mold. This investigation supports the contention that this could readily occur.

A generalized statement that all molds improve flavor would be erroneous but no more so than one to the effect that all molds are harmful and undesirable. Some of the molds, particularly some strains of Aspergillus, Rhizoctonia, Trichoderma and Fusarium produced very obnoxious flavors and odors.

Other molds, notably Mucor and Penicillium, after inoculation into the juice, imparted a sweet flavor during the first few days. After four days a taste panel preferred tomato juice containing the Penicillium strain to uninoculated juice.

The production of a high polygalacturonase potency by the molds which rapidly attacked tomato fruits in contrast to the absence or low PG potency associated with the molds which experimentally caused little damage, suggests that the enzyme polygalacturonase is responsible for much, if not all, of the damage done to tomatoes by some molds. The action of Rhizopus when introduced into tomatoes could certainly be ascribed to such enzymatic activity while the failure of Alternaria and Colletotrichum to behave in a similar manner could be explained by the lack of rapid PG production. PG assumed a similar role in the decomposition of black raspberries (White and Fabian, 1953), and in decomposition of strawberries (Beneke et al., 1954). One cannot overlook the degradative action which the molds might produce when allowed to develop in tomato tissue over a long period of time or under other conditions. These have not been considered in this study. Also, mass inoculation has been known to influence pathogenicity.

Additional studies of an extensive nature are required to develop greater information concerning the parasitism of fungi in tomato tissue.

Wright and Brian (1953) reported that they had isolated from some strains of Alternaria solani a product referred to

as alternaric acid. This had antifungal properties and was also markedly phytotoxic. Less than 1 p.p.m. on reinjection produced lesions in stems and leaves. However, no correlation was found between the pathogenicity of various strains of molds and their capacity to produce this acid. Oxalic acid produced by molds can not be disregarded (Valleau, 1915; Brown, 1948; Gibson, 1952).

Proteolytic enzymes might be considered for more detailed study (Wood, 1952).

The role of cellulase might be profitably investigated in greater detail. However, the low potency of cellulase produced by the molds employed in this study and the lack of relationship between it and the severity of attack supports the contention that it is of considerably less significance than polygalacturonase in the breakdown of tomato fruits.

SUMMARY

The principal genera and species of molds found in Indiana and Ohio tomato fruits were determined. The genera Oospora, Fusarium, Rhizopus, and Mucor were found to be more commonly associated with tomato defects than had been noted previously. By contrast, Alternaria solani and Colletotrichum phomoides, two widely distributed molds were found to be associated with only minor lesions in most instances, particularly in the early stages of attack. In addition to the molds commonly associated with tomatoes the genera Trichoderma, Mycelia and Hormodendrum were found.

Thirty-three cultures of the principal molds were inoculated experimentally into the tissue of sound whole tomatoes and the degree of attack was noted. Alternaria solani, Colletotrichum phomoides, Trichoderma sp. and Hormodendrum sp. produced relatively little damage to the tomato tissue compared to the action of species of Mucor, Oospora and Rhizopus. The latter was found to rupture tomato tissue and produce cracks in advance of the point of inoculation. The damage to tissue by the molds bore no relationship to the amount of visible growth.

Mold counts were made of tomatoes showing varying percentages of visible rot and it was determined that a percentage of visible rot as low as 0.1 percent could give a mold

count in excess of 50. The mold count associated with an established percentage of visible rot was markedly influenced by the genera of molds present. Average mold analysis could not be expected to know these molds, yet these molds are responsible for the severity of attack, flavor and odor changes.

It was emphasized that a low percentage of visible rot may mean Government seizure of pack while at another time this amount of visible rot would yield an acceptable Howard mold count.

The presence of the molds in tomatoes generally had the effect of making the tomatoes less acid. It was suggested that the increased alkalinity may improve the flavor of tomato juice yet promote the development of flat-sour spoilage. Comminuted tomato fruits containing some genera of molds were pleasant and in some instances showed improved flavor compared to fruits not containing mold.

The effect of factory procedures during tomato processing was studied. Trimmed tomatoes showed substantially higher mold counts than tomatoes which showed no blemishes and did not require trimming. This raised the question as to what a processor can do when soil and weather conditions encourage fungal attack. The answer appears to be for Federal, State and local agencies to encourage sound cultural practices. In the factory, it was suggested that careful inspection of final produce going into the product replace the Howard mold count.

A study was made of the production of polygalacturonase and cellulase by 120 molds when inoculated experimentally into tomato juice. The potency of polygalacturonase in wholly and partially decomposed areas trimmed from field tomatoes which had been invaded by various molds was determined. It was found that the ability of the molds to rapidly attack tomato tissue was coupled with the ability of these molds to produce polygalacturonase. The molds which experimentally produced the greatest amounts of PG were isolates of Mucor, Oospora and Rhizopus.

The majority of the molds isolated produced only a small amount of cellulase and this appeared to bear no relationship to the extent of decomposition associated with any of the molds.

It was emphasized that slight changes in the medium, the age of spores, the genus, the species and the strains of the mold markedly influence the ability of molds to invade or penetrate tomatoes. These and other factors suggest the need for additional studies of an extensive nature.

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