Populations and Softening Enzyme Activity of Filamentous Fungi on Flowers, Ovaries, and Fruit of Pickling Cucumbers[1]

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Although investigations have dealt with softening of brined cucumbers since 1899 (Aderhold), the studies have been primarily concerned with proving the bacterial theory of spoilage. Much effort has been directed toward implicating the aerobic bacilli as the responsible agents (Kossowicz, 1908; LeFevre, 1919; Fabian and Johnson, 1938; Faville and Fabian, 1949; Demain and Fabian, 1950, Unpublished Experiments; and Vaughn *et al.*, 1954).

The nature of the acid fermentation of cucumbers presents three important obstacles to serious consideration of the much-studied aerobic bacillus theory of softening spoilage: (1) the salt content of the fermenting brines; (2) the acidic condition of the brine and accompanying drop in pH resulting from early and rapid development of the lactic acid bacteria; and (3) the low oxygen tension of the brine attested to by excellent growth of the microaerophilic, lactic acid bacteria. Such conditions would indeed be highly restrictive if not wholly inhibitive for growth by aerobic, sporeforming bacteria of the genus *Bacillus*. Thus it is not surprising that populations for these organisms have not been demonstrated in cucumber fermentations brined under conditions typical of the industry.

Early brining studies (Unpublished) by Etchells and Jones (1940), conducted with replicated 45-gallon barrel lots at a commercial pickle plant in eastern North Carolina, confirmed the above conclusion that cucumber fermentations offer a rather unfavorable environment for development of aerobic bacilli. Heavy inocula of washed cells of young cultures of *Bacillus mesentericus fuscus* (*Bacillus subtilis*) failed to develop when added to cucumber fermentations at the outset, and the cured brine-stock showed no loss of firmness over uninoculated controls. Furthermore, in other experiments, broth filtrates from the above organism, when added to cucumber fermentations at the start, did not cause softening of the brined material.[5] This work, which employed a transfer of the same organism used by Fabian and Johnson (1938), was an attempt to extend to actual brining conditions the earlier experiments of these workers who concluded, on the basis of laboratory tests, that the aerobe, *B. mesentericus fuscus*, was chiefly responsible for the softening of brined cucumbers.

The various studies on the aerobic bacilli in relation to softening spoilage, beginning with Fabian and Johnson (1938), did much to reveal the nature of the pectinolytic enzymes elaborated by these organisms, particularly as to pH optima (above 7) but, in so doing, cast serious doubt as to their actual importance as the causative agents. For example, Fabian and Johnson found it necessary to "freshen" or desalt brine-cured cucumbers—thus removing both the salt and brine acid—before softening could be obtained with a filtrate from the growth of *B. mesentericus fuscus*. This, in effect, demonstrated that they were working, and probably the first to do so in the genus *Bacillus*, with a pectinolytic enzyme system with a high pH optimum (above 7) which would be inoperative at the pH range of regular brine-stock (4 and below).

Later Nortje and Vaughn (1953) established that the pectinolytic enzymes of *B. subtilis* (*B. mesentericus fuscus*) and *B. pumilis* had pH optima of 8.5 and 9.4, respectively. They also demonstrated the instability of the *B. subtilis* enzyme, 96 per cent was destroyed in 3 days at pH 4.5. These important findings would certainly further minimize the role of aerobic bacilli in softening spoilage. Observations based on numerous commercial cucumber fermentations in several important production areas of the United States have demonstrated that a rather precipitous drop in brine pH can be expected within a relatively short period after the vats are filled; for example, in the general range of pH 6.0 to 5.5 in 24 hr; 4.7 to 4.0 in 48 hr; and

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[5] Later, broth filtrates of this same culture were shown by Bell (1950, Unpublished Data) to possess a pectinolytic enzyme system with a pH optimum of 8.2; further, the enzyme was inactive below pH 7.0. These findings may explain the failure to obtain softening of the brined material in the aforementioned experiments. In this instance, the brine pH's of the fermentations were much below the activity range for a pectinolytic enzyme system such as that observed by Bell for *Bacillus mesentericus fuscus*.

below 4.0 in 3 to 4 days (Jones, 1940; Jones and Etchells, 1943; Etchells *et al.*, 1952; and Danielson, 1956, personal communication).

In 1950, Bell *et al.* concluded that the softening of 74 samples of commercially brined cucumbers from 19 brining stations located in nine states was the result of pectinolytic enzyme activity with a pH optimum of 4.0. Further, enzyme activity was found present in brines with a pH range of 3.2 to 3.9, salt content from 10 to 21 per cent (by weight) and brine acidity of 0.2 to 1.2 per cent expressed as lactic acid. Also, the brine enzyme was similar in behavior to purified polygalacturonase of fungal origin; the latter enzyme was able to soften firm, cured, cucumber brine-stock.

However, Vaughn *et al.* (1954) were of the opinion that aerobic, sporeforming bacilli, with their high pH optima pectinolytic enzyme systems (pH 8.5 to 9.4), might be responsible for enzymatic softening of commercially brined cucumbers, provided certain unusual conditions prevailed, that is, brine pH of 5.5 or above for several days and a predominant growth of aerobic bacilli to the near exclusion of the normal lactic acid bacteria. Such conditions in our experience would rarely exist. On the other hand, we observed serious outbreaks of softening spoilage in the southeastern states during 1947 and 1948, and in the North in 1955, in which as many as 150 vats of brine-stock softened despite early, vigorous lactic acid fermentations. Such occurrences call for a more satisfactory explanation; namely, that the softening enzyme systems in cucumber brines are chiefly of fungal origin as was first suggested by the work of Bell *et al.* (1950).

MATERIALS AND METHODS

Introductory Studies

During the 1951 growing season, work was started to determine the numbers and species of yeasts associated with different parts of the growing cucumber plant (*Cucumis sativus* L.). In the course of these studies it was readily apparent that a considerable flora of filamentous fungi was present on the various samples examined, particularly the flowers collected in the fields and those removed from immature cucumber fruit at the brining station (see table 1).

It was further noticed that, as the season progressed, fungus populations from material sampled from the same fields appeared to greatly increase (50 to 100 times). In fact, these populations were so high and growth was so luxuriant for the June 26th flower samples from four fields in Pitt County that yeast counts and isolations could only be made with considerable difficulty. More specifically, over one-half of the 34 samples collected on June 26th and July 2nd and plated or streaked on glucose agar could not be used for counting yeast populations or isolation of these organisms because of excessive fungal growth.

These first results provoked considerable discussion and conjecture as to the possible relationship of the fungal growth associated with flowers, particularly

TABLE 1

Origin, weight, and fungus populations of samples of cucumber material collected from ten fields and two brining stations located in two counties in eastern North Carolina—1951 Introductory Studies

Source	Type of Material Sampled	No. of Samples Collected*	No. of Flowers, Ovaries, or Fruit per Sample	Avg Wt		Fungus Colony Count per g†	
				Per sample	Per unit	Avg	Range
				g	g	$\times 10^3$	$\times 10^3$
Fields	Flowers						
	Staminate‡	18	25	3.36	0.134	19.9	1–170
	Pistillate‡	18	25	3.41	0.136	23.9	1–220
	Pistillate, unopened	1	25	2.10	0.084	4.0	—
	Ovaries	18	25	12.72	0.509	1.9	0.2–9.0
	Fruit§	1	18	137.0	7.6	3.0	—
Grading stations	Flowers¶	2	20	0.45	0.023	>1000.0	—
	Fruit§	4	18	153.5	8.4	2.4	0.4–4.0

* From four fields in Pitt County and six fields in Sampson County; two grading stations located in Pitt County, at Ayden and Bells Cross Roads. Four samplings made during the season, June 8 and 26 (Pitt County); June 14 and July 2 (Sampson County). Packer variety cucumber grown in Pitt County fields; Model variety grown in four of six Sampson County fields, York State in one and mixed varieties in one.

† Flower samples disintegrated in a Waring Blendor using 100 ml capacity stainless steel chamber; fruit and ovary samples, together with an equal weight of saline in 1 L and 25 ml flasks, respectively, shaken by hand (100×). Dilutions of blended flower samples and washings from fruit and ovary samples plated on dextrose agar and streaked with standard loop (0.01 g capacity) on poured plates of nitrogen base agar (see medium B, 1952 studies). Fungi counts represent average, seasonal values for all samples for both plating and streaking techniques.

‡ Freshly opened, collected in the morning.

§ Small, immature fruit, no. 1 size, approximately 5/8 in. diameter.

¶ Retained, partially dried flowers removed from no. 1 size machine-graded fruit.

those removed from fruit which normally would enter the brining vats, and enzymatic softening of commercially brined cucumbers. It seemed entirely possible that such growth and accompanying hydrolytic enzymes could be responsible for the pectinolytic activity first reported by Bell (1951) in staminate and pistillate flowers of two varieties of pickling cucumbers. In view of the rather serious limitations discussed earlier, connected with the aerobic bacillus theory of softening of brined cucumbers, the fungus-enzyme theory seemed both refreshingly different and plausible.

In this new approach to the origin of softening enzymes in brines, the responsible enzyme system concerned (chiefly pectinolytic) would be predeveloped, mostly as the result of fungal growth, and introduced per se into the brining vats, principally by flowers attached to immature cucumber fruit and to a lesser extent by the fruit itself. Such introduced enzyme would of necessity be required to have properties capable of softening activity under rather rigid conditions as to acidity, pH, and salt content offered by commercial curing brines. Pectinolytic enzymes attributed to fungal source meet these important requirements (Bell *et al.*, 1950); however, those of bacterial origin, such as produced by the aerobic sporeformers and the soft-rot bacteria that have been studied, apparently do not have these properties (Fernando, 1937; Oxford, 1944; Nortje and Vaughn, 1953; Kraght and Starr, 1953; Wood, 1955; and Demain and Phaff, 1957).

With the above points in mind, studies were designed and completed during the past few years to test the fungus-enzyme theory. These have dealt with microbiological, enzymological, and physical aspects of the softening problem, including: (1) microbiological flora (filamentous fungi, yeasts, and bacteria) of the growing cucumber plant with special reference to populations, taxonomy, and enzyme activity of the predominating fungal species; (2) observations under commercial conditions to determine the degree of retention of flowers on cucumber fruit throughout the harvest season, the enzyme activity of such flowers, and their ability to soften cucumbers in brining tests; (3) the use of certain control measures, such as the draining technique or the use of natural enzyme inhibitors, to significantly reduce softening enzyme activity in commercial brines; and (4) softening enzyme activity of cucumber flowers from northern and southern production areas of the United States.

Certain phases of the above investigation, namely those concerned with items 1, 3, and 4, have recently been reported (Etchells *et al.*, 1958; Bell and Etchells, 1958; and Bell *et al.*, 1958; Raymond, Etchells, Bell, and Masley, Unpublished Observations).

The present paper represents a detailed report on: population estimates of filamentous fungi occurring on flowers, ovaries, and fruit of the cucumber plant (*Cucumis sativus* L.); pectinolytic enzyme activity of such cucumber material; and pectinolytic and cellulolytic enzyme activity of certain of the fungus species isolated during the study. A summary of part of the work has appeared earlier (Etchells *et al.*, 1955).

Experimental Procedure

Collection of samples. During the 7-week period from June 5 to July 17, 1952, samples of cucumber flowers, ovaries, and immature fruit (figure 1) were collected at weekly intervals from five commercial fields, a brining station, and a greenhouse, all of which were located in eastern North Carolina. Information as to source of samples, type of material collected at each source, together with additional details as to sample size and total number of samples obtained, is given in table 2. All samples were collected using aseptic precautions, placed in sterilized paper bags by use of sterile forceps, labeled, refrigerated over night, and prepared for microbiological examination the following morning. Fruit samples and samples of retained flowers removed from this fruit collected at the brining station represented material typical of stock being sent from five receiving and grading stations operated by the cooperating company. A corresponding station was located in the vicinity of each of the five commercial fields that were sampled. The retained flower samples were pooled for each sampling interval because individual samples (20 flowers) from fruit from each receiving station weighed 0.40 g or less.

Preparation of samples. Individual flower samples from the fields and greenhouse (25 flowers each), after weighing, were placed in a sterilized, 50-ml capacity stainless steel Omni-mixer[6, 7] blending chamber, together with sufficient saline (0.85 per cent) to make a final dilution of 1:10. The mixture was then blended for 1 min at 14,000 rpm. Samples of partially dried flowers removed from no. 1 size fruit at the brining station were handled in essentially the same manner; however, because these samples (45 to 100 flowers) weighed considerably less than the fresh samples, a final dilution of 1:50 was found to be more desirable and convenient to handle in the 50 ml blending chamber. The weighed ovary samples (25 ovaries each) were transferred to 125-ml Erlenmeyer flasks and an equal weight of saline added. The flasks were placed on a rotary shaker[8] for 10 min at 210 oscillations per min.

[6] Basic power unit and stainless steel chambers with rotary knife blade sections were manufactured by Ivan Sorvall, Inc., Norwalk, Connecticut.

[7] It is not the policy of the U. S. Department of Agriculture or the North Carolina Agricultural Experiment Station to recommend the products of one company over similar products of any other company. The name supplied herewith is furnished for your convenience and information.

[8] Model V, capacity 40 flasks, manufactured by the New Brunswick Scientific Company, New Brunswick, New Jersey.

Figure 1. Left: Freshly opened pistillate flower typical of samples collected from the growing cucumber plant (*Cucumis sativus* L.) in commercial fields and in the greenhouse. For the purpose of this study, the pistillate flower, upon removal from the vine, was separated into two parts—the "flower" part and the "ovary" part.

Right: Partially dried flower retained on a small-sized (no. 1) immature cucumber fruit; this retained flower is typical of samples removed from 1 to 1⅛ in. diameter fruit at the brining station. The cucumber shown, after deflowering, is likewise representative of the fruit samples collected from fields and the brining station. About ¾ actual size.

The fruit samples (20 fruit each), together with an equal weight of saline in 1-L flasks, were shaken by hand (100 times).

Cultural and isolation procedure. Decimal dilutions of the blended flower samples and washings from the ovary and fruit samples were streaked on the surface of previously poured plates of three media by use of a calibrated platinum loop (0.01 g capacity). The media used were: (A) Littman's (1947) oxgall agar[9] with streptomycin (30 μg per ml) designed for primary separation and isolation of fungi from other microorganisms; (B) nitrogen base agar prepared from Wickerham's (1951) yeast nitrogen base[9] broth as described by Etchells *et al.* (1953) and acidified with 3 ml of 5 per cent tartaric acid per 100 ml of melted medium to inhibit bacteria, yet permit good growth of yeasts and adequate but restricted colonial development of higher fungi (Etchells *et al.*, 1954); and, (C) Conn's glycerol asparaginate agar (1921) used for cultivation of soil bacteria.

The streaked plates were usually incubated 4 days at 28 C and counted. At each plating interval, plates from the high dilutions of two media (A and B) were set aside for picking representative colonies of higher fungi directly to slants of potato dextrose agar. This resulted in a collection of 1032 fungus isolates for taxonomic study, which will be the subject of a separate report (Raymond *et al.*, Unpublished Observations) as will be the case for population studies of yeasts and bacteria. In addition to the taxonomic study, the proportion of each predominating fungus species present among the isolates was assigned to the

[9] Obtained from Difco Laboratories, Detroit, Michigan.

TABLE 2

Origin and weight of samples of cucumber flowers, ovaries, and immature fruit collected from five commercial fields, a brining station, and a greenhouse located in North Carolina—1952 season*

Source	Type of Material Sampled	No. of Places Sampled	No. of Samplings During Season†	No. of Samples of Each Type Collected	No. of Flowers, Ovaries, or Fruit per Sample	Avg Wt	
						Per sample	Per unit (i.e., flower, ovary or fruit)
						g	g
I, Fields	Flowers‡	5	7	35	25	3.51	0.140
	Ovaries	5	7	35	25	12.00	0.480
	Fruit§	5	5	25	20	265.4	13.3
II, Brining station	Flowers¶	1	5	5	45–100	0.5–2.0	0.017
	Fruit§	5‖	5	23‖	20	313.8	15.7
III, Greenhouse	Flowers‡	1	4	4	25	4.15	0.166
	Ovaries	1	4	4	25	9.68	0.387

* The five fields (1 to 1½ acres each of Packer variety) were located in eastern North Carolina in Pitt County and in the bordering counties of Craven and Lenoir. All fields were within a 20-mile radius of the brining station located at Ayden. The greenhouse was located at North Carolina State College, Raleigh; Model variety was grown in the greenhouse.

† Seven samplings from each of the five fields were made at intervals of 1 week as follows: June 5, 12, 19, 26, July 3, 10, and 17. No fruit available at the fields on June 5 and July 17. No material available at the brining station on June 5 and July 17. No greenhouse samples were available until June 26.

‡ For this study the freshly opened, pistillate flower was used (see figure 1).

§ Immature fruit, no. 1 size, approximately 1 to 1⅛ in. diameter (see figure 1).

¶ Retained, partially dried flowers removed from no. 1 size fruit (see figure 1).

‖ These samples consisted of fruit being sent to the brining station usually from five receiving and grading stations.

total colonies on the plate in order to obtain an estimate of their numbers in the samples collected throughout the season.

Pectinolytic activity measurements of samples. Certain dilutions of the blended flowers samples (1:100) and those of the washings from the ovary (1:10) and fruit samples (1:1), prepared for microbiological examination, were analyzed for pectinolytic activity by the method of Bell *et al.* (1950). In this procedure, the ability of a sample to decrease the viscosity of a 2.5 per cent pectin solution buffered with citrate at pH 4.0 and at 30 C is measured (100 units equals 50 per cent viscosity loss in 6 days).

Pectinolytic activity of fungi. Initially, a large number of isolates were rapidly screened by the technique of Bell and Etchells (1956). By this method, their ability to cause such biochemical changes of pectin during growth that would result in loss of gel properties is determined.

A total of 274 isolates, representing species of fungi in 34 genera and 10 unidentified groups, was screened; five cultural media,[10] containing 0.5 per cent pectin, were used for each culture tested. The reactions reported for the rapid screening technique in most cases are based on the combined observations from tests made using the five media; however, medium IVA, mineral salts plus pectin, was considered to give the most satisfactory results wherein positive or negative results were readily verified by pectinolytic enzyme activity measurements on the culture filtrates.

It is realized that with the rapid screening technique, the lack of pectinesterase production by an isolate may limit or prevent glycosidic hydrolysis by polygalacturonase. For this reason, pectinolytic activity of many of the isolates was further checked by the viscometric method of Bell *et al.* (1955). Here, the ability of the culture filtrate to reduce the viscosity of a 1.0 per cent sodium polypectate solution buffered with citrate at pH 5.0 and at 30 C is studied (100 units equal 50 per cent reduction in viscosity in 20 hr). The representative fungus cultures tested were usually grown for 2 weeks at 28 to 30 C in the mineral salts liquid medium of White and Downing (1951), supplemented with 0.5 per cent pectin.

Cellulolytic (C_x) activity of fungi. Assay of filtrates from cultures grown as described in the previous paragraph was carred out by the method of Bell *et al.* (1955). Using this procedure, 100 cellulolytic (C_x) units equal 50 per cent loss in viscosity in 20 hr of a 1.0 per cent sodium carboxymethylcellulose solution, buffered with citrate at pH 5.0 and at 30 C.

Other cellulolytic tests for fungi. The representative fungus cultures were examined for their ability to grow in a mineral salts broth with filter paper as the sole carbon source; also, certain of those shown to be positive were further tested for their cellulolytic activity by determining their power to destroy the tensile strength of cotton fabric. In the filter paper test, Whatman no. 4 paper was cut in 0.5 by 5 in. strips and placed one each as inverted U's in ¾ by 6 in. size test tubes, together with sufficient mineral salts broth (White and Downing, 1951) so that each strip was two-thirds submerged. The strips were inoculated from cultures growing on slants of potato dextrose agar by point inoculation with spores, or a bit of mycelium on both sides of the bend of the strip which extended above the liquid. Incubation was at 28 to 30 C at high humidity (90 per cent RH).

The procedure used for determining the ability of certain of the fungi to attack and reduce the tensile strength of cotton fabric in pure culture was essentially that described by White and Downing (1951). The exceptions were: (1) the use of 1 by 10 in. strips of 80 sq cotton print cloth (33 lb break-test after autoclaving) suspended as loops in 1 by 8 in. size test tubes containing 25 ml of mineral salts broth; and (2) the use of 7/16 in. width, special cotton label tape (50 lb break-test after autoclaving) suspended in the 1 by 8 in. size tubes with 25 ml of medium. The Instron Tester[11] was used for determining the tensile strength of all samples, including autoclaved, uninoculated controls.

[10] I, Pectin broth: peptone, 0.5 per cent; NaCl, 0.5 per cent; yeast extract, 0.25 per cent; glucose, 1 per cent; and pectin, 0.5 per cent; final pH 4.5. II, Nitrogen base broth (Wickerham, 1951): dehydrated N-base, 0.67 per cent; glucose, 1 per cent; and pectin, 0.5 per cent; final pH 3.8. III, Potato broth: potato broth, 20 per cent (200 g potatoes boiled 30 min in 800 ml water and the filtrate made up to 1 L); glucose, 1 per cent; and pectin, 0.5 per cent; final pH 4.3. IV, Mineral salts broth (White and Downing, 1951) plus glucose, 1 per cent, and 0.5 per cent pectin; pH 4.5, adjusted to 6.4. IVA, same as medium IV but without glucose.

[11] Model TT-B, manufactured by the Instron Engineering Corporation, Canton, Massachusetts.

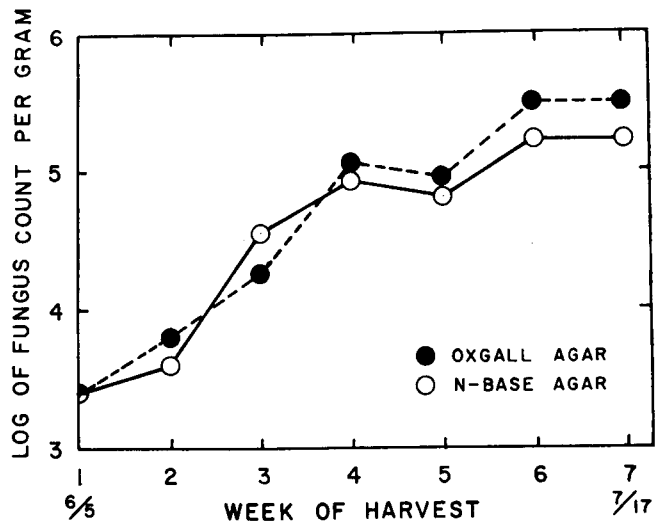

Figure 2. Population trends for fungi on cucumber flowers collected from fields throughout the season.

RESULTS

Remarks on cultural procedure. The streaking technique, with the selective media used, proved most satisfactory for estimating fungal populations of the various samples of cucumber material examined. In most cases, the colonies were well distributed over the surface of the plates and isolations for taxonomic purposes could readily be made without concern for contamination problems usually associated with picking subsurface colonies from samples plated on selective media. Two media, Littman's oxgall agar and acidified, nitrogen base agar, were used throughout the study. Both were generally considered adequate; however, there were some differences worthy of mention.

Population trends obtained with oxgall and nitrogen base agars were considered comparable, although slightly higher total numbers were usually obtained with the former medium (see figure 2). The oxgall agar plates required that strict attention be paid to counting and picking colonies within a 4-day incubation time; a longer period usually resulted in such luxuriant fungal growth as to preclude both counting and picking individual colonies. In contrast, the fungi developed more slowly on the nitrogen base agar, and the plates could be readily counted up to 10 days' incubation. The colonies, after this length of time, showed adequate growth (average, 1 to 1.5 cm in diameter), but were effectively restricted from spreading. This property of the medium permitted refrigeration of the plates until the conclusion of the study, and then the colonies were picked.

Some difficulty was encountered with streptomycin-resistant bacteria in the form of wet, spreading colonies growing on the oxgall agar plates. This was particularly noticeable with certain cucumber fruit samples collected from the brining station at the sixth sampling interval. In this instance, although fungus counts could be made for the five samples, bacterial contamination was such that fungus isolations could only be made from one sample. Restreaking of these samples with increased amounts of streptomycin (up to 100 μg per ml) did not control the contamination. Nitrogen base agar presented no bacterial problems; the pH of the medium (about 3.5) was sufficiently low to effectively control these organisms.

The foregoing discussion of the two media has considered certain cultural aspects connected with their use in enumeration and isolation of the fungi; any significant taxonomic differences between the isolates obtained from these media will appear in another report (Raymond *et al.*, 1958).

Fungus Population Studies

Flowers (see table 3). During the 7-week sampling period, fungus populations for freshly opened field flowers increased markedly, from 2500 per g to over 300,000 (see figure 2); essentially the same trend is obtained when calculated on a per flower basis (that is, 500 to 39,000). The average field flower weighed 0.140 g with a range of 0.090 to 0.181 g; the rather low values obtained from samples collected on June 26th and July 3rd were attributed chiefly to prevailing dry, hot weather.[12]

Fungus populations for partially dried flowers, removed from no. 1 size fruit at the brining station, were extremely high and reached 350 million per g toward

[12] The same explanation is offered for the noticeably lower counts obtained for all field and station samples collected on July 3rd; this sampling date coincided with approximate peak of the dry, hot weather conditions in the general area.

TABLE 3

Estimated populations of fungi from samples of flowers, ovaries, and fruit of cucumbers collected from different sources during the growing season

Source	Samplings During Season		Avg Unit Wt and Fungus Colony Count for					
			Flowers		Ovaries		Fruit	
	Week	Date	Unit wt	Count per g	Unit wt	Count per g	Unit wt	Count per g
			g	×10³	g	×10³	g	×10³
I, Fields								
	1	6/5*	0.181	2.5	0.504	1.3	—	—
	2	6/12	.162	6.5	.523	2.6	14.8	5.3
	3	6/19	.168	23.0	.590	4.0	10.7	5.6
	4	6/26	.107	110.0	.502	14.3	12.7	18.7
	5	7/3	.090	82.0	.375	9.6	12.8	11.2
	6	7/10	.152	325.0	.493	64.4	15.5	23.2
	7	7/17	.121	320.0	.372	36.7	—	—
Seasonal avg			0.140	124.1	0.480	19.0	13.3	12.8
II, Brining station								
	1	6/5*	—	—			—	—
	2	6/12	0.020	13,000			19.6	5.9
	3	6/19	.020	95,000			19.2	4.9
	4	6/26	.017	150,000			16.4	6.3
	5	7/3	.017	20,000			11.4	4.5
	6	7/10	.011	350,000			12.6	8.8
	7	7/17*	—	—			—	—
Seasonal avg			0.017	125,600			15.8	6.1
III, Greenhouse								
	4	6/26	0.152	6.0	0.384	3.3		
	5	7/3	.184	1.0	.404	0.6		
	6	7/10	.164	2.0	.344	0.5		
	7	7/17	.164	13.5	.416	3.5		
Seasonal avg			0.166	5.6	0.387	2.0		

* Minus sign in columns opposite this date means no material was available for sampling; also, no ovary samples were collected at the brining station and no fruit samples were available from the greenhouse.

Note: Fungus colony counts shown were obtained with oxgall agar.

the latter part of the season. The average station flower weighed 0.017 g (with a range from 0.011 to 0.020 g); this was about one-eighth the weight of an individual field flower. In view of the apparent weight difference, it is of interest to point out that the average station flower contained about 80 times the fungus population as compared to a field flower. This would indicate continued development of fungi in the flower during the time required for a fertilized ovary to grow to a no. 1 size fruit.

The low fungus count for greenhouse flowers is attributed mainly to the fact that flying insects were, for the most part, controlled. This was done by use of cheesecloth to cover the ventilator openings. Also, the greenhouse offered less opportunity for air-borne contamination as compared to open fields. The flower weights from this source were rather uniform during the period sampled (range of 0.152 to 0.184 g; average, 0.166).

Ovaries (see table 3). The ovaries from fields exhibited a similar upward trend for fungus populations during the season as was observed for field flowers, but the colony counts were of a much lower magnitude (about one-tenth). The average ovary weight was 0.480 g with a range from 0.372 to 0.590 g; the noticeable changes in weight at given sampling intervals correspond to those recorded for field flowers.

The fungus counts for ovary samples collected from the greenhouse were very low (average 2000 per g; 880 per ovary) and revealed no evidence of increased populations during the sampling period; the possible explanation for this finding has been discussed in connection with flowers from this source. Ovary weights from the greenhouse were fairly constant (0.344 to 0.416 g) and the average unit weight for the season was 0.387 g.

Fruit (see table 3). Fruit samples obtained from the fields demonstrated a moderate but definite increase in fungal counts during the season. However, fruit collected at the brining station remained rather constant with respect to population differences, and the seasonal average (6100 per g) was less than one-half that found for field fruit (12,800 per g).

One might expect the reverse of this situation to be found, considering the time required for field fruit to reach the brining station (about 6 to 8 hr) and the number of operations involved, that is, picking, bagging or boxing, transportation to the receiving station, sizing in a shaker-grader, and transportation to the brining station. These operations also help to rid the fruit of adhering soil which, in the present study, may account in part for the lower fungus counts from station fruit as compared to field fruit. Also, the dry weather may have been a factor in keeping spore germination to a minimum on fruit being sent to the brining station.

If calculated on an individual fruit basis, the fungus counts (presumably resulting mainly from spores on the fruit surface), shown in table 3, would be about 11 to 20 times higher, depending upon the average fruit weight for a given interval. The variability of fruit weights shown are to be expected for material collected in the approximate size range of 1 to 1⅛ in. diameter.

Pectinolytic Activity of Samples

Summarized results, based on seasonal averages, for softening enzyme activity of the various samples of cucumber material, together with seasonal fungus counts, are presented in figure 3. Additional information pertinent to the study of enzyme activity of samples collected from different sources during the season follows.

Flowers collected from fields, or from the brining station, were extremely high in pectinolytic activity; this was particularly the case for samplings subsequent to June 19th. Further, flowers from four of the five individual fields were generally comparable as to the amount of enzyme activity found throughout the season; samples from one field were consistently lower

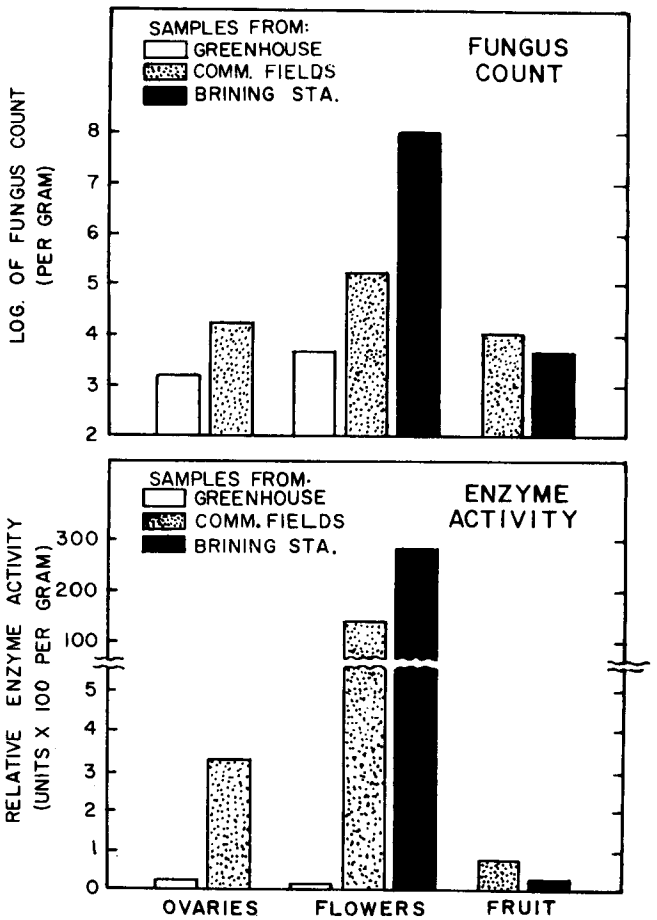

Figure 3. Seasonal fungus counts (*upper part*) and pectinolytic enzyme activity (*lower part*) of samples of cucumber material collected from three sources during the season.

in activity than the others. Sample area comparisons for station flowers cannot be made because enzyme activity was determined on a pooled sample for each collection date.

Pectinolytic activity of ovaries and fruit collected from fields was considerably lower than that of either station or field flowers; such activity was primarily restricted to material from the June 26th and July 3rd samplings. Also, of the five fields sampled, material from three contributed practically all of the pectinolytic activity for ovary and fruit samples.

Fruit collected from the brining station had about one-fourth the pectinolytic activity as compared to field fruit; further, the former was consistently low in enzyme activity throughout the season. Samples of flowers and ovaries collected from the greenhouse gave little evidence of pectinolytic activity; six of the eight samples collected during the season gave negative readings; two, one each for flowers and ovaries, were very low.

Maximum pectinolytic activities for samples collected from fields and from the brining station were reached coincident with the June 26th sampling. These values (table 4), together with those from greenhouse material, permit comparison of peak enzyme activities obtained for different types of samples.

Pectinolytic activities of cucumber material, such as those shown in table 4 and those in figure 3 were measured at pH 4.0. This would indicate that such enzyme activity was of fungal origin. This view is further supported by the high fungus counts obtained for station and field flowers which correlated very well with their high enzyme activities; also, station fruit gave low fungus populations, together with enzyme activity of a low order. Finally, the greenhouse samples revealed low fungus populations and negligible enzyme activity.

The low pectinolytic activity for field ovaries and fruit samples from the last part of the season did not correlate too well with the fungus populations. This could be attributed to a predominance of accumulated fungus spores on the ovary and fruit surface during this period. Such spores would develop as colonies on the streaked plates, but provide little activity for the enzyme test as compared to mycelial growth. It also should be mentioned that the fungus flora could change with the result that less pectinolytic species might predominate on field fruit and ovaries during the latter part of the season.

TABLE 4

Maximum pectinolytic activities for samples collected from fields and from the brining station

Sample (June 26)	Pectinolytic Activity Units	
	Per g	Per flower, ovary, or fruit
Station flowers	>50,000	>4200
Field flowers	>40,000	>8500
Field ovaries	1,300	650
Field fruit	245	3100
Station fruit	30	460
Greenhouse ovaries	0	0
Greenhouse flowers	0	0

Softening of Commercially Brined Cucumbers

Judging from the extremely high softening enzyme concentration of station flowers, as shown in table 4 and figure 3, it would seem most likely that if sufficient amounts were introduced into curing vats, under suitable conditions, softening of the stock would result. This apparently was the case, because, of a total of 13 commercial vats of no. 1 size cucumbers brined by the cooperating company during the 1952 season, between June 14th and July 12th, 10 were rated as being soft on October 30th by the plant manager. Further, 5 of the 10 vats with soft stock were filled during the period of peak softening enzyme activity for station flowers, June 26th.

Subsequent studies, presented by Etchells *et al.* (1955) and covering several seasons at two commercial plants, have substantiated the above relationship of cucumber flowers to enzymatic softening of brined cucumbers. Vats filled with small-sized cucumbers that had a high percentage of flowers, either retained naturally or added experimentally, usually resulted in brine-stock that was soft or inferior in firmness; the degree of softening obtained was dependent upon the enzyme concentration of the flowers. In contrast, when cucumbers were deflowered by hand, the cured brine-stock was exceptionally firm.

Action of Fungi on Pectin and Cellulose

Over 1000 cultures of fungi were isolated from flowers, ovaries, and fruit of the cucumber plant during the 1952 season. This collection of isolates was subjected to taxonomic investigation, and the results will be reported in detail by Raymond *et al.* (Unpublished Observation). Inasmuch as representative isolates of various identified species were tested for their action on pectin and cellulose, a brief summary of the taxonomic findings, based on the above report, is of interest.

Of the 1032 cultures isolated, 964 (93.4 per cent) were reduced to 72 species in 34 genera; the remaining 68 isolates (6.6 per cent) were placed in 10 unidentified groups. Further, 31 species in 5 genera represented 80.5 per cent of the total isolations, and 41 species in 29 genera account for 12.9 per cent. Five single species in the 5 genera—*Penicillium*, *Ascochyta*, *Fusarium*, *Cladosporium*, and *Alternaria* represented 60 per cent of all isolations. These species were: *Penicillium oxalicum*, 15.7 per cent of the isolates; *Ascochyta cucumis*, 15.4 per cent; *Fusarium roseum*, 10.3 per cent; *Cladosporium cladosporioides*, 10.1 per cent; and *Alternaria tenuis*,

TABLE 5

Pectin degradation by fungi isolated from cucumber material as assayed by the rapid screening test and by the viscometric method

Fungus	Screening Test			Viscometric Method		Previous Reports
	No. of isolates			No. of isolates tested	Pectinolytic activity rating*	
	Obtained	Tested	Positive			
MAJOR GROUP (A)† (and per cent of isolates)	(831)					
Fusarium (24.1%)	(249)					
F. roseum	106	9	8	3	4+, 4+, 2+	P;‡ *g*
F. oxysporum	60	11	11	2	4+, 2+	P; *d*
F. solani	58	7	7	2	3+, 3+	P; *g*
F. moniliforme	10	3	3	2	4+, 3+	P; *g*
F. episphaeria	1	1	1			
Fusarium sp.	14	0§				
Penicillium (22.3%)	(230)					
P. oxalicum	162	19	19	2	4+, 4+	P; *d*
P. janthinellum	16	10	10	2	4+, 4+	P; *h*
P. steckii	11	4	4			
P. expansum	8	3	3			P; *a*
P. sublateritium	7	3	3			
P. nigricans	5	0§				
P. frequentans	1	1	1			P; *a*
14 Other species¶	21	21	21			
Ascochyta (15.5%)	(160)					
A. cucumis	159	15	15	2	4+, 3+	
Ascochyta sp.	1	1	0			
Cladosporium (10.1%)	(104)					
C. cladosporioides	104	28	28	2	4+, 3+	
Alternaria (8.5%)	(88)					
A. tenuis	88	34	34	2	4+, 3+	P; *a*
MINOR GROUP (B)‖	(133)					
Aspergillus						
A. elegans	1	1	1			
A. fumigatus	7	3	3	1	2+	P; *a*
A. niger	1	1	1	1	2+	P; *a, c, d, e, f, h*
A. sydowi	1	1	1			
A. terreus	1	1	1			P; *d*
A. unguis	1	1	1			
A. versicolor	2	2	2			P; *a*
Cephalosporium						
Cephalosporium sp. 1	5	4	2	1	1+	
Cephalosporium sp. 2	1	0§				
Cercospora sp.	1	1	0			
Chaetomium						
C. globosum	2	2	0	2	N, N	N; *a*
Choanephora						
C. cucurbitarum	4	0§				
Colletotrichum						
C. lagenarium	8	2	2	1	3+	
Cunninghamella						
C. echinulata	1	1	0	1	N	
Curvularia						
C. trifolii	4	4	4	1	3+	
Geotrichum sp.	3	3	0	1	N	
Gliocladium						
G. roseum	2	1	0	1	N	
Helminthosporium sp.	2	2	2	1	3+	
Heterosporium						
H. terrestre	1	1	0			
Humicola						
H. fusco-atra	1	1	1	1	2+	
Metarrhizium						
M. anisopliae	2	2	0	1	N	

TABLE 5—*Continued*

Fungus	Screening Test			Viscometric Method		Previous Reports
	No. of isolates			No. of isolates tested	Pectinolytic activity rating*	
	Obtained	Tested	Positive			
Mucor						
M. silvaticus	19	3	3	1	2+	
Mucor sp. 2	1	1	1			
Mucor sp. 3	1	1	1	1	2+	
Myrothecium						
M. roridum	4	3	1	1	1+	
M. verrucaria	9	6	0	1	N	
Myrothecium sp.	2	2	1			
Papularia						
P. arundinis	1	1	1	1	N	
Pestalotiopsis						
P. macrotricha	3	2	2	1	1+	
Phoma sp.	7	3	3	2	4+, 2+	
Pullularia						
P. pullulans	3	3	3	1	1+	
Pseudonectria sp.	1	1	0			
Rhizopus						
R. arrhizus	1	1	1			P; *b*
R. nigricans (= *R. stolonifer*)	4	2	2	1	1+	P; *b*
Rosellinia sp.	1	1	0			
Sporotrichum						
S. pruinosum	3	2	2	2	1+, 1+	
Stagonospora sp.	1	1	1			
Stysanus sp.	1	1	0	1	N	
Thielavia						
T. basicola	3	3	3	1	1+	
Trichoderma						
T. viride	16	15	5	2	3+, 2+	P; *a*
Truncatella sp.	1	1	1			
UNIDENTIFIED:	(68)					
Sporulating fungi	48	7	4			
Sterile fungi	20	10	4			
Control broth	0			N		

* Based on activity of clear fungus culture filtrate after 14 days' growth: 4+ = very strong activity, approximately 500,000 pectinolytic units per ml; 3+ = strong, approximately 100,000 units; 2+ = moderate, approximately 25,000 units; 1+ = weak, 1,000 to 10,000 units; N = negative activity, <10 units.

† Genera and species listed in decreasing order of frequency of isolation; group A fungi represent 80.5 per cent of total isolations (1032); group B, 12.9 per cent; unidentified, 6.6 per cent.

‡ P means positive for pectinolytic activity and N means negative as reported in the literature as follows: *a*, Dingle and Solomons (1952); *b*, Harter and Weimer (1921); *c*, Popova (1935); *d*, Ragheb and Fabian (1955); *e*, Saito (1955); *f*, Schubert (1952); *g*, Vaughn *et al.* (1957); *h*, Waksman and Allen (1933).

§ Cultures dead.

¶ Isolates in the following 14 species were all positive in the rapid screening test: *P. atromentosum*, 1 isolate; *P. chermesinum*, 1; *P. duclauxi*, 2; *P. funiculosum*, 2; *P. gladioli*, 1; *P. herquei*, 1; *P. implicatum*, 1; *P. piscarium*, 2; *P. restrictum*, 2; *P. rugulosum*, 1; *P. thomii*, 2; *P. urticae*, 1; *P. variabile*, 2; and *Penicillium* sp., 1.

‖ Genera and species listed alphabetically.

8.5 per cent. If the above list is extended to the 10 most frequently isolated species, the 5 following species would be included: *Fusarium oxysporum*, 5.8 per cent of isolates; *F. solani*, 5.6 per cent; *Mucor silvaticus*, 1.8 per cent; *Penicillium janthinellum*, 1.6 per cent; and *Trichoderma viride*, 1.6 per cent.

The results for the action of representative cultures of the various species of fungi on pectin and cellulose are presented in tables 5–7. It will be noted that most of the species tested proved to be both pectinolytic and cellulolytic. This was particularly true for cultures representing the most frequently isolated species. Additional information concerning this portion of the study follows.

Action on pectin. A total of 274 isolates was tested for pectin degradation by the rapid screening procedure (table 5). Of these, 170 were typical of species in the 5 most frequently isolated genera, group A; 104 were representative species in 29 less frequently obtained genera, group B. Of the 29 species tested in group A,

27 were completely positive for degradation of pectin, 1 was negative, and 1 species appeared to be variable (8 isolates positive out of 9 tested). Of the 39 species tested in group B, 23 were positive, 11 were negative, 3 were variable, and 2 were doubtful.

Verification of the action on pectin described above was made by measuring the pectinolytic activity of culture filtrates from representative species (table 5). Isolates typical of species in group A were highly pectinolytic, with the exception of 1 isolate each of *Fusarium roseum* and *F. oxysporum*, which were moderate in activity. Of the 25 species of group B tested, 8 were negative, 7 were weak, and the remaining 10 ranged from moderate to very strong in pectinolytic enzyme activity. The two assays of pectin breakdown checked well; the only discrepancy was in the case of *Papularia arundinis*, which was positive in the rapid screening procedure but whose culture filtrate failed to reduce the viscosity of the polypectate solution.

When our results, shown in table 5, are compared to the results of previous workers, it can be seen that no disagreements are in evidence. It is further noted that species in many of the fungi genera apparently have not been studied heretofore.

Action on cellulose. Extensive studies by White *et al.* (1948), Marsh *et al.* (1949), and Reese *et al.* (1950) have been made on the cellulolytic activity of large collections of fungus isolates representing numerous species in a wide variety of genera. In these important investigations, activity was measured by decline in tensile strength of cotton fabric resulting from growth of pure cultures of the fungi in a mineral salts medium.

Based on these reports, the following general patterns of cellulolytic activity for the 34 genera of fungi obtained from the cucumber plant seem likely: (1) activity would be expected with a high degree of frequency among isolates of 18 genera, that is, *Alternaria*, *Cladosporium*, *Cephalosporium*, *Chaetomium*, *Fusarium*, *Curvularia*, *Gliocladium*, *Helminthosporium*, *Humicola*, *Myrothecium*, *Papularia*, *Pestalotiopsis*, *Sporotrichum*, *Trichoderma*, and possibly *Phoma*, *Heterosporium*, *Thielavia* and *Stysanus*; (2) negative results for activity would be expected for isolates of 6 genera, that is, *Cunninghamella*, *Geotrichum*, *Metarrhizium*, *Mucor*, *Pullularia*, and *Rhizopus*; (3) activity among isolates of *Aspergillus* and *Penicillium* would not be expected to occur with any degree of regularity except for certain species in the various groups, subgroups, or series of these genera; and, (4) it seems that an insufficient number of isolates have been studied in the 8 remaining genera, *Ascochyta*, *Cercospora*, *Choanephora*, *Colletotrichum*, *Pseudonectria*, *Rosellinia*, *Stagonospora*, and *Truncatella*, to establish generic cellulolytic activity patterns.

The isolates listed in table 5, under Viscometric Method, were tested for ability to grow with filter paper as the sole source of carbon. All showed moderate to heavy growth, with the exception of the following 8 species in genera belonging to group B: *Colletotrichum lagenarium*, *Cunninghamella echinulata*, *Geotrichum* sp., *Metarrhizium anisopliae*, *Mucor silvaticus*, *Mucor* species no. 3, *Pullularia pullulans*, and *Rhizopus nigricans*. Furthermore, growth of the positive isolates was

TABLE 6

*Cellulolytic activity of 19 species of Penicillium on cotton tape after 10 days' growth in a mineral salts medium**

Fungus	Culture Number	Growth Index†	Loss in Tensile Strength	Enzyme Activity of Culture Filtrate‡	
				Cellulolytic units/ml	Pectinolytic units/ml
(19 *Penicillium* species)	CF-		%	×10	×10
P. atromentosum...	733	5+	90	180	39
P. chermesinum...	250	4+	80	220	15
P. duclauxi.....	1018	4+	75	240	13
P. expansum.....	116	1+	0	<10	3
P. frequentans.....	983	0	0	<10	3
P. funiculosum....	891	4+	75	1580	98
P. gladioli.....	743	3+	90	1020	80
P. herquei.....	9	3+	75	440	17
P. implicatum.....	1007	0	0	<10	4
P. janthinellum...	224	5+	95	1640	240
P. janthinellum...	317	5+	90	1160	327
P. janthinellum...	422	5+	90	4000	227
P. janthinellum...	753	5+	90	1000	117
P. janthinellum...	830	4+	95	2480	227
P. oxalicum.....	8	5+	95	260	77
P. oxalicum.....	134	5+	95	320	480
P. oxalicum.....	326	5+	90	180	62
P. oxalicum.....	405	5+	90	340	10
P. oxalicum.....	519	5+	90	780	19
P. oxalicum.....	600	5+	90	460	387
P. oxalicum.....	1057	5+	90	560	187
P. piscarum.....	1	4+	60	110	72
P. restrictum.....	747	1+	0	<10	3
P. rugulosum.....	934	2+	0	20	2
P. steckii.....	782	0	0	<10	3
P. sublateritium...	62	4+	90	860	37
P. thomii.....	667	5+	60	1000	4
P. urticae.....	378	0	0	<10	3
P. variabile.....	936	2+	0	460	3
(5 Comparison species)					
Myrothecium verrucaria.....	580	4+	100	440	5
Chaetomium globosum.....	490	4+	90	200	3
Aspergillus niger..	129	3+	30	680	3
Trichoderma viride.....	1056	3+	25	180	13
Mucor species.....	36	1+	0	<10	17
Sterile control..		0	0	<10	3

* Based on unpublished studies by Templeton, Etchells, Bell, and Campbell (1955).

† 5+ = Very heavy growth; 4+ = heavy; 3+ = moderate; 2+ = slight; 1+ = very slight; 0 = no visible growth.

‡ Inoculated, toluene preserved controls were made for each organism tested; all gave negative results for loss in tensile strength of tape and enzyme activity of culture filtrate.

accompanied by cellulase (C_x) activity in the clear culture filtrate with the exception of *Penicillium oxalicum* (CF-8) and *Cladosporium cladosporioides* (CF-31).

It is of interest to note that of the 8 negative cultures, 7 belong to the 6 genera that would be expected to give negative results for cellulolytic activity; the remaining isolate (CF-419), representing *Colletotrichum lagenarium*, apparently has not been studied in this respect. Of the 30 cultures showing growth on filter paper, practically all belonged to genera in which cellulolytic activity has been observed with a high degree of regularity.

Cellulolytic activity of Penicillia (see table 6). Nineteen species of *Penicillium*, together with five comparison species from other genera, all isolated from cucumber material, were tested for their ability to degrade cotton tape. Eleven *Penicillium* species, including all isolates of *P. oxalicum* and *P. janthinellum*, proved to be active in reducing the tensile strength of the test material and showed C_x activity in the broth filtrate. The most cellulolytic *Penicillium* species were comparable in activity to *Myrothecium verrucaria* and *Chaetomium globosum*, which are two of the most cellulolytic fungi known. Certain species, notably all isolates of *P. janthinellum* and certain of the *P. oxalicum* isolates, produced considerable pectinolytic enzyme activity during growth on the cotton tape. Eight species were inactive as to degradation of the tape and, with one exception, were considered negative for C_x activity; *P. variabile* showed 4600 units per ml of C_x activity, 2+ growth on the tape, but no loss in tensile strength.

The results for nine *Penicillium* species listed in table 6 (that is, *P. oxalicum*, *P. janthinellum*, *P. expansum*, *P. frequentans*, *P. funiculosum*, *P. restrictum*, *P. rugulosum*, *P. thomii*, and *P. variabile*) agree with earlier observations by Marsh *et al.* (1949); these workers studied numerous *Penicillium* isolates, including all the species shown in table 6 except *P. piscarium*. Lack of agreement in the tests for the nine remaining species common to both studies could readily be attributed to variation in biochemical behavior of isolates of the same species. This was demonstrated by the above workers with three isolates of *P. funiculosum*; two were strongly cellulolytic and one was inactive. However, cellulolytic activity seems to be rather strong for certain species; the seven isolates of *P. oxalicum* and five of *P. janthinellum* were uniformly highly active, with values for loss in tensile strength of cotton fabric of 90 to 95 per cent; Marsh *et al.* obtained values of 93 to 94 per cent for these two species.

Of the five comparison species, all were positive for tape degradation with the exception of the *Mucor* isolate. These results agree perfectly with their ability to grow with filter paper as sole carbon source. In addition, several isolates each of *Penicillium oxalicum*, *P. janthinellum*, *Chaetomium globosum*, *Myrothecium verrucaria*, *Trichoderma viride*, *Cladosporium cladosporioides*, *Ascochyta cucumis*, *Sporotrichum pruinosum*, and *Mucor silvaticus* were tested for their ability to

TABLE 7

Pattern of pectinolytic and cellulolytic activity of the 10 most frequently isolated fungi from flowers, ovaries, and fruit of the cucumber plant

Fungus	No. of Isolations and Percentage of Total (1032)		Action of Representative Isolates on:					
			Pectin		Filter paper		Cotton fabric	Cucumbers
			Rapid screening test	Pectinolytic activity	Growth	Cellulolytic activity (C_x)	Loss of tensile strength	Loss in firmness*
	no.	%						%
Penicillium oxalicum	162	15.7	+	+	+	+	+	100
Ascochyta cucumis	159	15.4	+	+	+	+	+	70
Fusarium roseum	106	10.3	+	+	+	+	+	30
Cladosporium cladosporioides	104	10.1	+	+	+	+	+	70
Alternaria tenuis	88	8.5	+	+	+	+	+	80
Fusarium oxysporum	60	5.8	+	+	+	+	+	95
Fusarium solani	58	5.6	+	+	+	+	+	90
Mucor silvaticus	19	1.8	+	+	−	−	−	30
Penicillium janthinellum	16	1.6	+	+	+	+	+	100
Trichoderma viride	16	1.6	V†	+	+	+	+	90
Total	788	76.4						

* The fungi were grown for 3 weeks at 30 C in medium IVA (mineral salts with pectin) and 1 ml each of the clear fungus filtrates were added to quart jars of commercially prepared, pasteurized cucumbers (1 in. diameter, Model variety) equalized at 2.5 per cent salt, 0.6 per cent acetic acid and pH 3.7; all jars, including controls, were preserved with toluene, resealed, and incubated 18 days at 30 C. The U.S.D.A. Fruit Pressure Tester was used to determine cucumber firmness (Bell *et al.*, 1955); controls without added filtrates averaged 15.5 lb whereas cucumbers from jars with purified polygalacturonase (1 mg per quart jar) showed complete loss of firmness.

† Variable.

degrade cotton cloth. All were active except *M. silvaticus*.

Pattern of enzyme activity of principal fungi. A probable pattern of pectinolytic and cellulolytic activity of the 10 most frequently isolated fungi from flowers, ovaries, and fruit of the cucumber plant is shown in table 7. It will be observed that all of these species, which represented almost 75 per cent of the total isolations (1032), are pectinolytic and all but one are also cellulolytic. Further, they were active in reducing the firmness of cucumber tissue. The first five species listed accounted for 60 per cent of the isolates obtained, and in addition composed most of the total fungus populations of field flowers and station flowers throughout the 1952 season. In view of this, it seems most likely that growth by these particular species was chiefly responsible for the concentration of pectinolytic activity demonstrated for such flower samples. It will be recalled that the softening of 10 commercial vats of brined cucumbers during the 1952 season was attributed to pectinolytic enzyme activity of cucumber flowers adhered to the cucumber fruit introduced into the curing vats.

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Summary

Results are presented for a study on: population estimates of filamentous fungi occurring on samples of flowers, ovaries, and fruit of the cucumber plant (*Cucumis sativus* L.) throughout one growing season; pectinolytic enzyme activity of such cucumber material; and pectinolytic and cellulolytic enzyme activity of representative fungus species isolated in the course of the investigation.

During the 4- to 6-week harvest season, rather high fungus populations and accompanying pectinolytic enzyme activity were obtained for certain samples of cucumber material. This was especially true for flowers collected from fields and those removed from fruit at the brining station. The latter, because they are introduced into the vats with the fruit that is brined, were considered a potent source of softening enzymes in commercial cucumber brines.

Representative isolates of the various species of fungi isolated from cucumber material, subsequently identified in a companion study, were tested for their action on pectin and cellulose. Of the 72 species in 34 genera obtained, most proved to be both pectinolytic and cellulolytic; this was particularly the case for cultures representing the most frequently isolated species.

Five species in five genera, *Penicillium*, *Ascochyta*, *Fusarium*, *Cladosporium* and *Alternaria*, represented 60 per cent of all isolations (1032) and were found to be highly important sources of pectinolytic enzyme activity. Growth by these particular species was considered to be chiefly responsible for the fungal populations and pectinolytic enzyme activity demonstrated for flower samples collected during the season.

The studies reported definitely implicate filamentous fungi as the actual causative agent responsible for softening-spoilage of cucumbers brined under commercial conditions typical of the South. Further, it is believed that the softening enzyme systems are introduced into curing brines chiefly by way of the fungus-laden flowers that remain attached to the cucumbers, and to a lesser extent by the fruit itself.

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