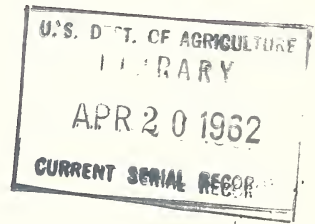


Ag 847m
Cap. 3

Alternaria Rot Following Chilling Injury of Acorn Squashes



Marketing Research Report No. 518

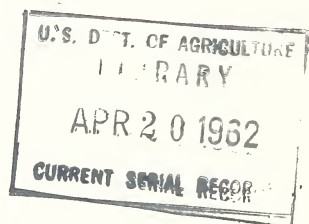
UNITED STATES DEPARTMENT OF AGRICULTURE
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Contents

	Page
Summary.....	4
Review of the problem.....	5
Materials and methods.....	6
Response to storage temperatures.....	7
Color changes.....	7
Kinds of decay found.....	7
The dependence of alternaria infection on low-temperature injury.....	7
Natural occurrence of alternaria rot.....	7
Inoculation studies.....	10
Tolerance of the <i>Alternaria</i> fungus to low temperature.....	12
A study of the <i>Alternaria</i> fungus.....	13
<i>Alternaria</i> infection and decay.....	13
Description of alternaria rot.....	14
Identification of the <i>Alternaria</i> fungus.....	15
Literature cited.....	19

Summary

Acorn squashes were physiologically weakened by chilling storage for 25 days or longer at 32° and 40° F. Chilling injury was not manifested by physiological symptoms, but was indicated by alternaria rot that developed during post-storage holding at 65°. The period required for chilling injury varied, but generally was 20 to 25 days at 32°, and 25 to 30 days at 40°. Squashes were injured more at 32° than at 40° and the injury increased as the storage period was lengthened. Squashes stored at temperatures of 45° and above for 35 days did not develop alternaria rot. Those stored at 45° for 60 days became chilled and developed alternaria rot.

The species of *Alternaria* isolated from squashes was identified as *Alternaria tenuis* auct. The mycelium and spores both grew at temperatures as low as 27° F. *Alternaria tenuis* is compared with *Alternaria cucumerina* in this report. Spore measurements are given for each and both are illustrated in order to identify *A. tenuis* as the cause of squash-fruit rot.

The predominance of alternaria rot on squashes following low-temperature storage is directly related to an abundant natural spore load, ability of the fungus to grow at low temperatures, and the weakened condition of squashes due to chilling injury. This combination permits the weak pathogen *Alternaria* to appear as a vigorous and important pathogen.

Alternaria Rot Following Chilling Injury of Acorn Squashes

By LACY P. MCCOLLOCH, *senior plant pathologist*, Market Quality Research Division, Agricultural Marketing Service ¹

Review of the Problem

This study was prompted by the suspicion of low-temperature injury in a commercial shipment of acorn squashes which developed a high percentage of alternaria rot. The numerous spots of alternaria rot on the squashes were so strikingly similar to those found on low-temperature-injured tomatoes (8)² that chilling injury was immediately suspected.

Past research on winter squashes has dealt with long-term cold-storage as a possible means of decay control. However, as far as is known, there are no reports on the effect of short-term refrigeration on acorn squashes either in transit or in storage.

The merits of long-term cold storage of squashes as a method of decay control were considered by certain early investigators, who generally concluded that storage at low temperature increased decay. Guba and Gilgut (5) were encouraged to try low-temperature storage because they found that certain squash-rotting fungi failed to grow on culture media, during the period tested, at 40° F. and below. Yeager and associates (12) found that certain decays which developed at high temperatures did not occur on squashes stored at low temperatures. They attempted to control decay by storing squashes at 32° to 42°, but several years' results, demonstrated to them that low-temperature storage was not feasible. Cummings and Jenkins (1), working with small lots of Hubbard squashes, found weight loss least at average temperatures of 36° and 39°. All of the squashes decayed, however. Cummings and Jenkins considered the decay to be associated with a sudden drop in storage temperature rather than with gradual low-temperature injury. Platenius and Jamison (10) reported extensive decay in squashes stored at 35° to 40° for 3 to 5 months.

¹ Acknowledgment is made to the following members or previous members of the Market Quality Research Division: John T. Worthington, Cleveland Brown, William Ebersole, and Kenneth Wisner, who assisted in conducting the investigation; and Paul L. Lentz, of Mycology Investigations, Crops Research Division, for technical assistance in reviewing the status of *Alternaria cucumerina*.

² Italic numbers in parentheses refer to Literature Cited, p. 19.

They placed the emphasis on high humidity rather than on low temperature as the contributing factor. Yeager and associates (12) reported that Blue Hubbard squashes could not be saved from decay at 32° and 40° even with a relative humidity of 40 percent.

These investigators sought to find the optimum storage conditions for squashes. In general, they did not discuss the effects of low temperature other than to point to the extensive decay that followed. Most research workers now agree that decay of squashes is greatly increased by storage at low, but nonfreezing, temperatures.

This study is part of a broad program of research aimed at reducing marketing costs.

The purposes of the present study were to determine the time-temperature relationship to chilling injury of acorn squashes; determine symptoms of chilling injury; relate the occurrence of alternaria rot to chilling injury; identify the species of *Alternaria* responsible for the rot; and obtain information on the nature and pathogenicity of the *Alternaria* fungus.

Materials and Methods

Acorn-type squashes of the Table Queen variety were grown at Beltsville, Md., in 1954, 1955, and 1957 for time-temperature studies in relation to chilling injury and subsequent alternaria rot. Crops grown in 1958 and 1959 were used for a study of symptoms of alternaria rot and for inoculation studies with the *Alternaria* fungus. The crop was harvested at normal maturity between September 14 and 24 to avoid low temperatures in the field. The squashes were washed and graded for size, shape, soundness, and maturity. Well-developed fruits with a dark green color were judged to be of proper maturity. Experimental lots were carefully selected for uniformity and included in the test within a day after harvest.

Test lots were stored at 32°, 40°, 50°, 55°, 60°, and 70° F., for 20, 25, 30, and 35 days and then removed from storage and held at 65° for 6 to 10 days. In 1957 extra lots were stored at 32° and 45° for 60 days. The relative humidity of the storage rooms was higher than that recommended for long-stored winter varieties of squashes. The conditions were similar, however, to those used for short-term storage of fresh produce in which acorn squashes are sometimes included. Examinations were made as each lot was withdrawn from storage and again after the holding period at 65°. Data were obtained on decay and physiological deterioration.

Small lots of acorn squashes from other localities were occasionally purchased in order to observe the kind of decay that developed following storage.

Materials and methods employed in other phases of this study are discussed in the section concerned.

Response to Storage Temperatures

Color Changes

The storage life of acorn squashes is relatively short. The color began to change from a desirable dark green, which denotes freshness, to yellow at 50° F. in 35 days. Those stored at 55°, 60°, and 70° for the same period were undesirably yellow and the flesh was becoming stringy. The dark-green color of those at 45° remained practically unchanged during 35 days of storage. Yellowing did not occur at 40° and 32° but the squashes were injured by these temperatures.

Kinds of Decay Found

Decay that developed during post-storage holding at 65° F., was influenced by mechanical and insect injuries of the squashes in the test and by the storage temperature and period.

Black rot *Mycosphaerella citrullina* ((C.O. Sm.) Gross.) accounted for most of the decay in squashes stored at 50° F. or above. It also developed at 65° as a secondary rot on fruits previously held at 32° and 40°. Black rot was greatly increased by pickleworm and mechanical injuries.

An occasional instance of gray mold rot (*Botrytis cinerea* Fr.), fusarium rot (*Fusarium* sp.), and mucor rot (*Mucor* sp.) was found. The most consistent and striking pattern of decay, however, was alternaria rot on squashes stored at 32° and 40° F.

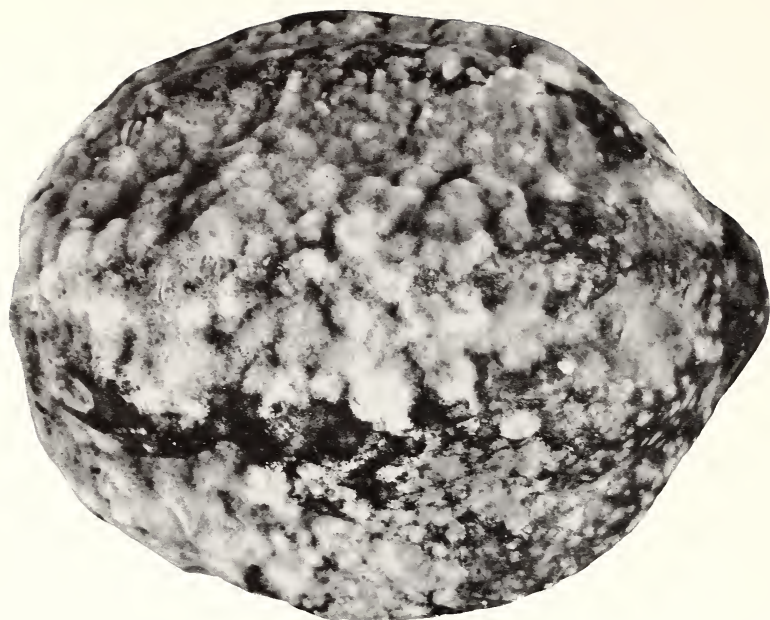
The Dependence of Alternaria Infection on Low-Temperature Injury

Natural Occurrence of Alternaria Rot

Acorn squashes were physiologically weakened by chilling storage for 25 days or longer at 32° and 40° F. Chilling injury did not cause any symptoms of a physiological nature. The injury was indicated, however, by the development of alternaria rot during a 6- to 10-day holding period at 65° following low-temperature storage. Squashes were injured much more at 32° than at 40°, and the number of alternaria lesions per fruit developed in proportion to the extent of injury (figs. 1 and 2). The apparent soundness of squashes as they were removed from storage at low temperatures was deceptive. Infection took place during storage at 32° and 40°, but visible decay had not developed in 35 days. About 55 days were required for typical decay to develop at 32°.

Squashes stored at 45° F., or above for 35 days, were not injured and remained sound (fig. 3). Squashes held at 45° for 60 days or longer were injured, however, and developed alternaria rot at that temperature.

The minimum period required for acorn squashes to be injured and to become susceptible to *Alternaria* varied somewhat from season to



BN-15012

FIGURE 1.—*Alternaria* rot, from natural infection, following chilling injury. Acorn squash held at 32° F., for 35 days, then at 65° for about 1 week. Note the numerous lesions.



BN-15011

FIGURE 2.—*Alternaria* rot, from natural infection, following chilling injury. Acorn squash held at 40° F., for 35 days, then at 65° for about 1 week.



BN-15010

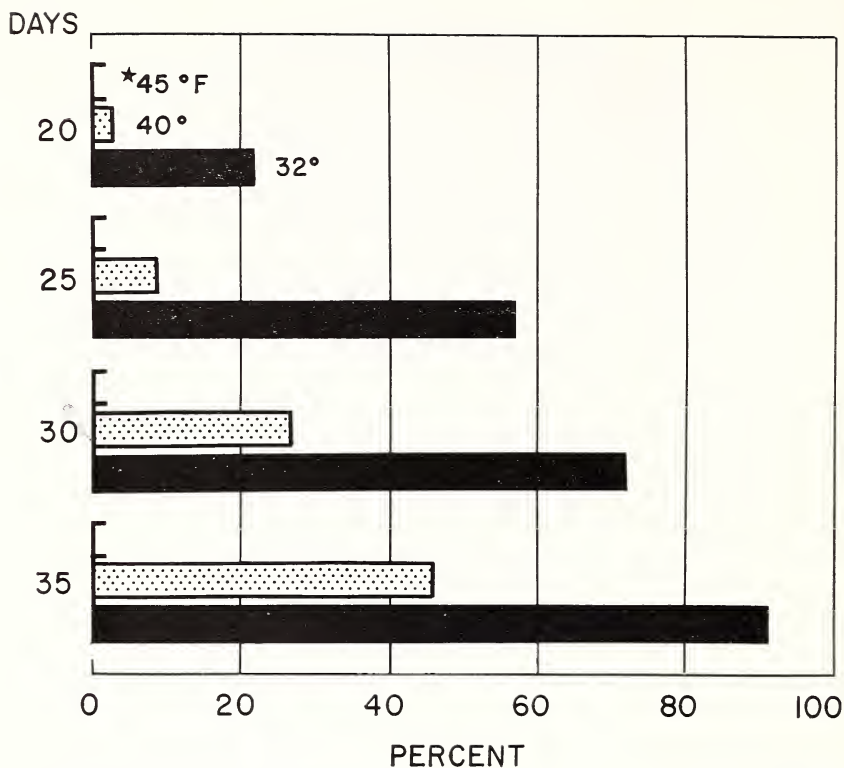
FIGURE 3.—Acorn squash held at 45° F., for 35 days, then held at 65° for 6 days. The sound condition was typical of squashes with this treatment.

season. One year 6 percent of the squashes developed alternaria rot at 65° F. following a 15-day storage period at 32°. Usually, however, 20 to 25 days at 32° or 25 to 30 days at 40° were necessary to weaken the squashes enough for them to become susceptible. The longer they were subjected to low temperature the more susceptible they were to alternaria rot.

The striking effect of low temperature in predisposing squashes to alternaria rot is seen by comparing those stored at 45° F. (there was no alternaria rot at 45° or above) with those stored at 32° and 40° (fig. 4). The data are an average of three seasons' tests, and were obtained following a 6- to 10-day post-storage period at 65°.

Further evidence that chilling injury is the predisposing factor in alternaria rot of acorn squashes is shown in figure 5. For comparison, the percentage of squashes that developed alternaria rot in around 1 week at 65° F., following storage at various temperatures, is plotted on the same figure with data on rate of growth of the *Alternaria* fungus on agar at temperatures of 30° to 80° F.

The growth rate of *Alternaria* on cornmeal agar at temperatures between 30° F. and 80° was a typical temperature response. Growth developed slowly at 32° and 40°, but increased steadily with each 5 degree rise in temperature until the optimum was reached. The exact optimum was not determined, but the best growth at those temperatures tested (77°, 78°, 79°, 80°, and 85°) occurred at 80°. Although not included in figure 5, growth was less at 85° than at 80°. The rate of growth at the near-optimum of 80° was used as the base for determining the rate of growth at other temperatures.



*No decay at 45°.

BN-15009

FIGURE 4.—*Alternaria* rot that developed at 65° F., following storage at 32°, 40°, and 45°.

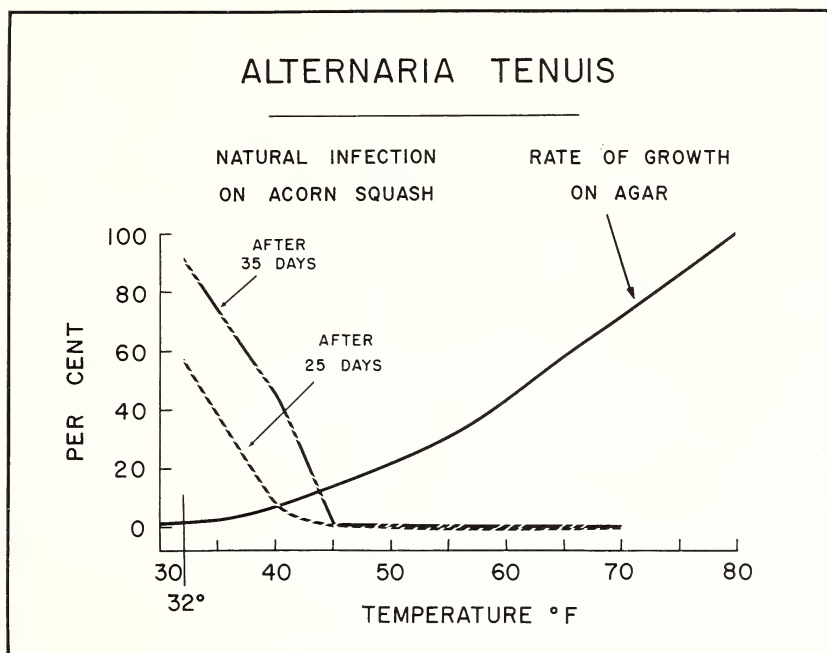
The temperature response of the *Alternaria* fungus is typical of that of many other fungi. The tendency has been to assume that the pattern of decay occurrence parallels the growth rate on agar, with the greatest hazard of decay being near the optimum temperature. This study shows that such a generalization does not apply to the *Alternaria* fungus. For example, squashes remained entirely free of alternaria rot at temperatures most favorable to the growth of the fungus, and infection occurred at temperatures unfavorable for growth of the fungus.

Inoculation Studies

The purpose of inoculating squashes was to determine whether those subjected to low temperatures were more susceptible to alternaria rot, as a result of chilling injury, than nonchilled squashes. Dark-green squashes free of blemishes were used. Each lot was carefully selected for uniformity as to size, shape, and color.

The *Alternaria* fungus was grown on cornmeal agar. Cultures about 7 days old were used. Several variations in inoculation techniques were tried. In techniques where squashes were wounded in connection with inoculation, the area was swabbed with 95-percent alcohol.

ALTERNARIA TENUIS



BN-15008

FIGURE 5.—The rate of growth of *Alternaria tenuis* on agar increased steadily between 32° and 80° F., reaching an optimum at about 80°. The reverse was true for alternaria rot on squashes. The highest percentage of rot occurred in those stored at 32° for 35 days, and no alternaria rot occurred on squashes stored 35 days at temperatures of 45° to 70°.

Following inoculation the inoculum was covered by damp cotton pads and the squashes were held in fiberboard trays enclosed in ventilated polyethylene bags.

The first test was with unwounded tissue. A 4-mm. disk of inoculum was inverted over the unwounded epidermis on squashes previously held at 32°, 40°, 45°, and 50° F., for 25 days. After 10 days at 65° the *Alternaria* had grown through the cotton and was sporulating. The fungus was unable to penetrate the unbroken skin, however, and no infection occurred. A similar test was made in which a 4-mm. disk of inoculum was inverted over a single needle puncture on each squash. No infection occurred after incubation under moist cotton pads for 10 days at 65°, even though the fungus grew through the cotton.

Four tests were made in which the squashes were held at 32°, 36°, 40°, 45°, and 50° F., for 25 days and then inoculated. A flap-type surface wound was made with a 4-mm.-diameter cork borer held nearly parallel to the squash. A 4-mm. disk of inoculum was inserted under the flap in contact with the exposed flesh. The inoculum was kept moist as indicated earlier and the squashes were held at 50° F. for 16 days. The wound-inoculation techniques followed by a prolonged holding period under ideal conditions of moisture provided very favorable environment for the fungus. It grew through the cotton pads, but decay on squashes initially held at 32°, 36°, and 40° F., for 25 days was limited and there was no active rot on those

held at 45° and 50° (table 1). The inoculum infected the wounded tissue so slowly that the site of inoculation was often decayed from natural *Alternaria* infection before the inoculated tissue could become decayed on squashes injured by holding at 32°, 36°, and 40°. Much of this problem was corrected in the last two tests by placing the inoculum on the underside of the squashes, in flap-type wounds, where there was very little natural inoculation.

TABLE 1.—*Acorn squashes stored for 25 days at temperatures indicated then inoculated with Alternaria and incubated at 50° F., for 16 days*

Storage temperature (° F)	Squashes inoculated	Average diameter of lesions
	Number	Millimeters
50-----	41	¹ 5. 1
45-----	41	¹ 4. 7
40-----	41	9. 0
36-----	39	12. 2
32-----	37	15. 7

¹ Slight tissue breakdown but no active rot.

Tolerance of the *Alternaria* Fungus to Low Temperature

Data in previous sections demonstrated that the occurrence of *alternaria* rot was dependent on low-temperature injury. The question of why *Alternaria* is the predominant cause of decay in chill-injured squashes prompted a study of the tolerance of the *Alternaria* fungus to low temperatures. The purpose was to determine whether the spores of *Alternaria* could germinate or whether mycelium could make measurable growth at temperatures of 32° F., and below. Wiant's (11) *Alternaria* isolates made a growth of 6 to 12 mm. in diameter at 35° in 3 weeks. Studies of the effects of 32° or below, however, have not been reported.

Isolate S-40 was used for this study. In the rate of growth studies a 4-mm. disk of inoculum was planted in the center of 90-mm. diameter petri dishes supplied with about 20 ml. of cornmeal agar. Dishes of inoculated agar were immediately placed at 32°, 30°, and 27° F. A minimum of eight dishes was used at each temperature.

These studies revealed a surprising tolerance of *Alternaria* to low temperatures. At an average temperature of 32° F. (range 31.5° to 34°), diameters of 5, 19, and 28 mm. developed in 12, 60, and 90 days. At an average temperature of 29.9° (range 29° to 33°) diameters of 5, 9, and 13 mm. developed in 24, 60, and 90 days. At an average temperature of 26.9° (range 24° to 31°) diameters of 4.5 and 11.0 mm. developed in 90 and 270 days, respectively. At 26.9° the agar in the dishes soon froze, and was frozen when the dishes were removed at the end of the holding period. The agar was permitted to thaw and the *Alternaria* made new growth during 2 days at room temperature.

Germination counts were made on *Alternaria* spores at 31.7°, 29.4°, and 27.3° F. in a further study of low-temperature tolerance. Cultures were grown on water agar and the spores floated free in about 10 ml. of sterile water. Strips of sterile lens paper, about ½ by 1½ inches, were dipped in the spore suspension, dried lightly, and placed in sterile petri dishes on solidified water agar. Upon making contact with the agar the paper strips immediately absorbed moisture, thus favoring spore germination. Five petri dishes were placed at each of the three temperatures and the test was repeated twice.

Extra samples at each temperature provided material to observe. Data were obtained when about 75 percent or more of the spores had germinated. Germination counts were made by transferring the spore-seeded lens paper to glass slides. Spore visibility was aided somewhat by staining the lens paper with methylene blue in 25 percent alcohol. Counts were then made on germinated and dormant spores. The results show (table 2) that the spores germinated freely at 27.3° to 31.7° F. if given sufficient time. The agar was frozen at 27.3° when the dishes were removed and was probably frozen most of the time.

TABLE 2.—*Germination of Alternaria spores at low temperatures*

Temperature			Spores observed	Period for 75 percent to germinate
<i>Average</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Number</i>	<i>Days</i>
31.7	31.5	31.8	393	5
29.4	29.0	29.5	275	10
27.3	26.0	28.0	415	20

A Study of the *Alternaria* Fungus

Alternaria Infection and Decay

The predominance of alternaria rot on squashes following low-temperature storage is directly related to an abundant natural spore load, ability of the fungus to grow at low temperatures, and to the weakened condition of squashes due to chilling injury. This combination permits the weak pathogen *Alternaria* to appear as a vigorous and important one.

The presence of a natural load of *Alternaria* spores on acorn squashes at harvest was demonstrated repeatedly by the numerous colonies of decay that developed naturally on squashes stored at 32° F. (fig. 1). The spores were lodged mostly on the upper surface and the decay was largely confined to that area. The side of the squash that rested on the soil was relatively free of alternaria rot.

A microscopic study of the earliest lesions showed that the lenticels served as infection courts.

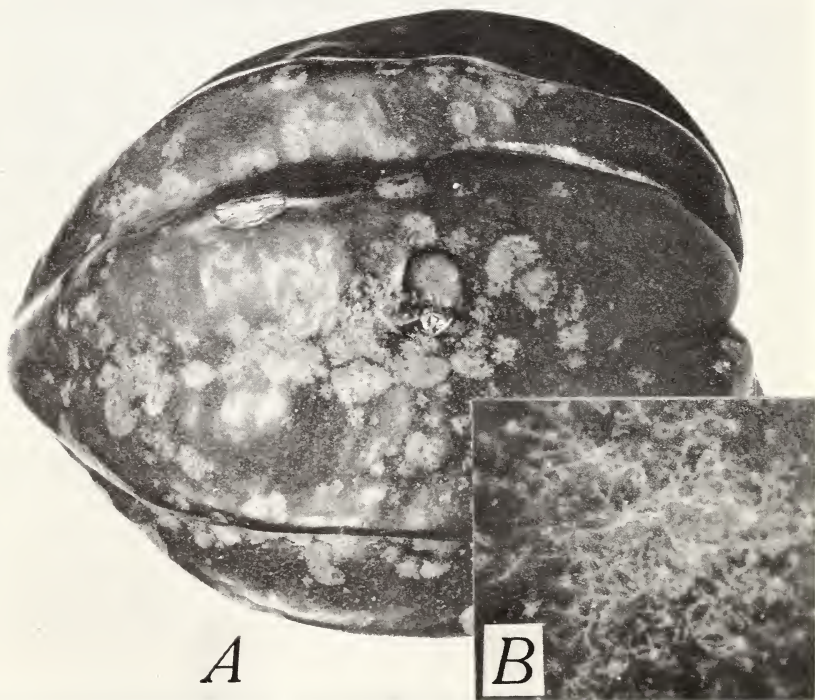
It required about 40 days at 32° F. for visible lesions to appear, and about 50 days for well-developed decay. Visible lesions developed at

40° in about 25 to 30 days. The percentage of fruits with visible decay increased sharply after 4 days at 65°.

Description of *Alternaria* Rot

The earliest stage of visible *alternaria* infection was characterized by pale cream-colored or bleached areas in contrast to the normal dark green color of acorn squashes. Affected areas were very superficial and were more or less circular but not sharply defined (fig. 6-A). Individual spots during the very early stages had a rhizoidal appearance due to the arrangement of colorless cells. The colorless cells extended from the center of affected lenticels and appeared as many branched strands forming a distinctive pattern (fig. 6-B). In seriously affected squashes the spots coalesced and formed large areas which were usually quite bleached.

After removal from 32° and 40° to 65° F., decay progressed rapidly. Decayed tissues below the surface first appeared somewhat bleached, soft, and mealy, but later became blackish and relatively firm. The rot was most easily recognized after surface mold developed over the lesions. The surface color was medium gray if the mycelium prevailed or a velvety olive green if spore masses predominated. Eventually the surface color became quite dark. The number of lesions was dependent on low-temperature injury.



6A—BN-15007

6B—BN-15006

FIGURE 6.—Early stage of *alternaria* rot, following cold storage, characterized by: A, bleached areas with irregular margins; and B, enlargement of spot showing rhizoidal pattern.

Identification of the *Alternaria* Fungus

Most investigators of the storage of winter squashes and other cucurbits have reported alternaria rot as a cause of spoilage at or following low-temperature storage. In general, the causal fungus was identified only as *Alternaria* sp.

In studying the market diseases of cantaloups, Honey Dew, and Honey Ball melons, Wiant (11) attempted specific identification. He examined 21 *Alternaria* isolates and found considerable variation in cultural characteristics. The spore sizes, however, fell within a range of 10μ to 60μ by 8μ to 22μ , and averaged 25.4μ by 11.9μ . Wiant considered the possibility of the leaf-spot fungus, *Alternaria cucumerina* (Ell. & Ev.) J. A. Elliott, being the causal fungus of the melon rot. He discarded this possibility, however, because the spores as previously described were longer, wider, and more tapering than the spores of his isolates.

Wiant did not illustrate the spores of his isolates, but was of the opinion that they could be placed within either the group typified by *Alternaria tenuis* Nees or that typified by *A. brassicae microspora* (Berk.) Sacc. Wiant's studies were made prior to the work of Neergaard (9) and that of Groves and Skolko (3), and lacking this guide to the nomenclature of *Alternaria*, he limited the identity of his isolates to *Alternaria* sp.

In 1950 Guba (4) published an extensive report on "Spoilage of Squash in Storage." He discussed alternaria rot of Hubbard squashes under the heading of black spot. He attributed the squash-fruit rot to *A. cucumerina*. Guba did not give measurements of the spores of his fungus, and the fungus was not available for study. Spores illustrated in figure 4 in his publication (4) are not typical of *A. cucumerina*. The drawings, however, possess characteristics similar to *A. tenuis*.

In an effort to clarify the specific identity of the *Alternaria* that causes rot of stored squashes a comparison of *A. cucumerina* and the isolates reported in this study was undertaken. The original description by Ellis and Everhart (2) was reviewed with a hope that their description would sharply delimit *A. cucumerina*. Their description, is, in part, as follows: "Conidia clavate, slender stipitate, 3-8 septa, scarcely constricted, submuriform, $30-75\mu \times 15-25\mu$, pedicel, $25-35\mu$ long. Nearly allied to *Macrosporium solani* E. & M. but differs in its slender pedicillate, mostly smaller conidia." They measured the body of the conidia separately from the beak, which they referred to as the pedicel. This has caused confusion in the literature for in all instances where the dimensions of the conidia of *A. cucumerina* have subsequently been stated the length of the body only has been given. Even when the lengths of the body and the beak of the conidia, as given by Ellis and Everhart, are summed, the overall length is too short for conidia of this type.

Because of this inadequate description, attention was then directed to the herbarium specimen. The material examined was part of the type collection in Ellis and Everhart North American Fungi, specimen No. 3396 on file in the National Fungus Collection, Beltsville, Md. Conidia with beaks intact were mounted in weak KOH, measured, and photographed. The range found in 42 conidia was $90-275\mu \times 15-25\mu$ and the average was $187.4\mu \times 21.9\mu$. Typical conidia with long, slender, tapering beaks are shown in figure 7. The illustration



BN-15005

FIGURE 7.—Conidia of *Alternaria cucumerina* prepared from herbarium specimen No. 3396.

and the measurements of the conidia, taken from the original type collection immediately serve to sharply delimit *A. cucumerina* from the isolates in this study.

Alternaria isolates from acorn squash grown in Texas, Florida, New Jersey, and Maryland were examined. All had similar characteristics yet there were the usual variations between isolates. They bore long chains of conidia, often branched, which showed greater variation within the members of the chain than between isolates. From the beginning the isolates were thought to be identified with *Alternaria tenuis* (fig. 8).

Measurements were made on conidia from five isolates obtained during the 1957 storage tests. Although single-spore lines are of little value in studying a fungus as unstable as *A. tenuis*, this was done as a matter of standard procedure. The isolates were grown on water agar to suppress vegetative growth and to stimulate sporulation. Conidia were measured when cultures were 5 to 10 days old, and before secondary characters had developed in the conidia. Measurements were made at random, excepting conidia with less than two cross septa. These were rejected as being improperly developed. A minimum of 150 conidia were measured from each isolate. Distribution curves showing the variation found in length and diameter of conidia between isolates are included in figures 9 and 10. The average length and diameter and the range in length and diameter of conidia from each isolate are shown in table 3. Except for the extremes in length and diameter of a few conidia (figs. 9 and 10) the majority readily came within the range suggested by Mason (6). The isolates in question are therefore considered as biotypes included in the elastic concept of *Alternaria tenuis* auct., the species responsible for alternaria rot of stored squashes.



BN-15004

FIGURE 8.—Conidia of *Alternaria tenuis* isolated from acorn squash.

VARIATION IN LENGTH OF CONIDIA OF ALTERNARIA ISOLATES

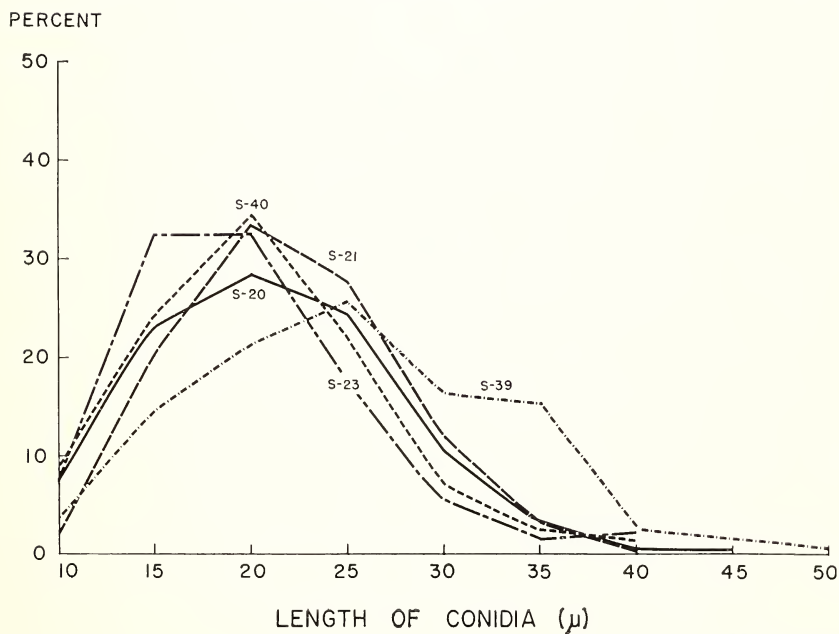


FIGURE 9

AMS-170-62(4)

VARIATION IN DIAMETER OF CONIDIA OF ALTERNARIA ISOLATES

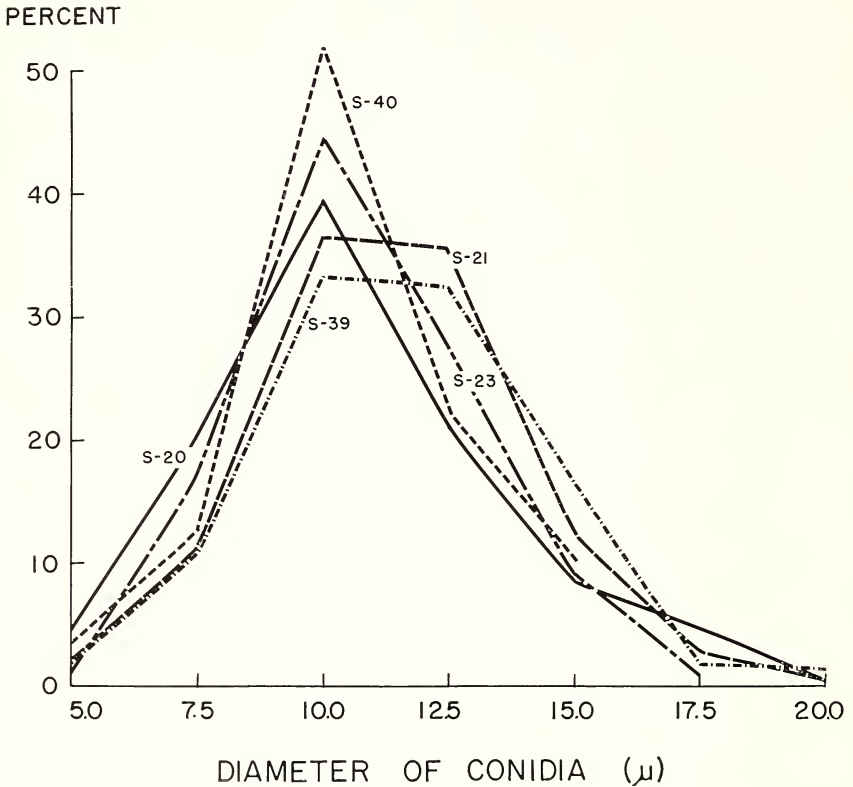


FIGURE 10

AMS-171-62(4)

TABLE 3.—*Measurements of conidia of five Alternaria isolates from decay of Maryland-grown acorn squashes*

Isolate No.	Conidia measurements	
	Average	Range
S-20	<i>Microns</i> 22.0 x 11.0	<i>Microns</i> 10.0—45.0 x 5.0—19.0
S-21	23.2 x 11.9	12.5—40.0 x 5.5—18.0
S-23	20.9 x 12.5	10.0—44.0 x 6.5—17.5
S-39	26.0 x 12.2	10.0—60.0 x 5.5—20.0
S-40	21.7 x 11.0	10.0—40.0 x 6.0—16.0

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Growth Through Agricultural Progress