Prevalence and Pathogenicity of Fungi Associated with Achenes of Sycamore in the Field and in Storage

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ABSTRACT

The most prevalent fungi associated with sycamore achenes were species of *Alternaria, Aureobasidium, Cladosporium, Epicoccum, Fusarium, Pestalotia, Peyronellaea, Phoma, Phomopsis,* and *Xylaria.* They varied in prevalence with the location, physiographic region, and year of collection.

Achenes stored at 2 C showed no loss in germinability even after 7 months. At 20 and 30 C, however, germinability decreased with increasing temperature, relative humidity, and time in storage. Most fungi isolated from achenes at the time of harvest decreased in prevalence with increasing temperature, relative humidity, and time in storage. At the same time, *Aspergillus ruber, Aspergillus repens,* and *Nodulisporium hinnuleum,* fungi that had not been isolated from the achenes prior to storage, increased in prevalence with time in storage.

Surface-disinfected achenes containing 15% moisture (wet weight basis) were inoculated with spores and mycelia of five fungi and stored at 30 C and 84-86% relative humidity. Those inoculated with *Aspergillus ruber* and *Aspergillus repens* decreased in germinability more than noninoculated controls after storage for 35 days. Phytopathology 61:660-668.

Additional key words: Platanus occidentalis, angiosperm.

Postharvest deterioration of various food and fiber crops is an increasingly important aspect of plant pathology. Recently, substantial progress has been made in understanding the nature of losses in quality and germinability of seeds of many agricultural crops (2, 3). Much has been learned empirically about moisture content (MC) and temperature conditions that will prevent losses in germinability during storage of seeds of many trees (7). Comparatively little is known, however, about the role of fungi in causing these losses. Although some research has been done on the fungi associated with seeds of gymnosperms (1, 6, 9, 10, 14), knowledge of the fungi associated with and causing injury to seeds and seedlings of angiospermous trees is incomplete (7, 10, 11, 15, 16, 17).

Comprehensive studies of fungi associated with seeds of higher plants, their ecological relationships, and their influence on the germinability and quality of seeds have been made with the cereal grains (3). The fungi involved have been classified into two major groups, "field fungi" and "storage fungi".

This investigation was made to determine if similar types of organisms are associated with achenes of sycamore (*Platanus occidentalis* L.), a typical angiospermous tree species. Specific objectives were to determine (i) the prevalence of fungi associated with sycamore achenes in the southern United States; (ii) the influence of storage conditions on the MC, germinability, and populations of fungi associated with sycamore achenes; and (iii) the capacity of the fungi isolated most commonly during the survey and storage studies to injure seeds during storage.

MATERIALS AND METHODS.—Sycamore fruit balls were collected in February and March 1967 at nine locations in three physiographic regions: two Mountain locations, near Vonore, Tenn., and Asheville, N.C.; four Piedmont locations, near Roxboro and Raleigh, N.C., Catawba, S.C., and Athens, Ga.; and three Coastal Plain locations, near Tarboro, N.C., Summerville, S.C., and Bogalusa, La. An additional study was made with achenes collected in 1967, 1968, and 1969 at three locations in North Carolina, near Roxboro, Raleigh, and Tarboro. The achenes were collected directly from standing trees grown in forests at each location except Raleigh, where achenes were obtained from forest trees and city trees.

Moisture content of achenes was determined by the 105 C-16-hr oven method recommended by the International Seed Testing Association (8), except that 3-4 g rather than 4-5 g of mixed achenes (achenes with and without seeds) were used. Results were expressed as percentages of the original (wet) wt of the achenes.

Seed germinability was determined both in lots of achenes and full achenes (achenes with seeds). Full achenes were identified by visual inspection for a swelling at their narrow end. Dissection of these achenes confirmed that they do contain seeds. One hundred mixed or full achenes were placed on sterile filter paper in sterile, glass petri dishes containing 10 ml of distilled water and incubated at 28 ± 2 C under fluorescent light of about 4,000 m candles (lux). Germinability was expressed as the percentage of achenes from which radicles were visible after incubation for 14 days.

Fungi present in the achenes were isolated from surface-disinfected mixed achenes and from achenes

that were dissected into pericarp and seed, then surface-disinfected. Mixed and full achenes were submerged in 1% NaOCl for 1 min, pericarps for 30 sec, and seeds for 4-5 sec; all were rinsed 3 times in sterile distilled water before being placed on the two media used for isolation. The potato-salt medium (PSM) contained 5 g Difco potato extract, 20 g dextrose, 17 g Difco agar, 60 g NaCl, and distilled water to give 1 liter of medium with a pH of 6.2 after sterilization at 121 C for 20 min. The rose-bengal medium was prepared as described by Tsao (12) for RBM-2, and had a pH of 6.5 after sterilization at 121 C for 20 min. Achenes, incubated on these two media at room temperature (23-28 C) were examined at a magnification of ×25 every other day for 20 days. When possible fungi were identified in the petri dishes, others were subcultured for later identification. Whenever possible, fungi were identified to species. Prevalence of fungi was expressed as the percentage of achenes, pericarps, or seeds yielding each fungus.

Achenes collected in 1968 from city trees in Raleigh and from forest trees near Tarboro, N.C., were stored at three temperatures (2, 20, and 30 C), three relative humidities (RH) (74-76, 79-82, and 84-88%), and four storage times (0, 2, 4, and 7 months). The entire experiment was repeated 3 times.

The three humidities were maintained in sealed 4-liter glass jars containing saturated solutions of NaCl, $(NH_4)_2SO_4$ and KCl, respectively. Six 120-ml, wide-mouthed glass bottles containing about 12 g of achenes from each source were supported about 4 cm above the salt solutions. Temperatures were controlled by placing each jar in a water bath inside a temperature-controlled incubator. Each replication was maintained in a separate set of three incubators. After each storage time, MC and seed germinability of part of the achenes were determined. Fungal isolations also were made from surface-disinfected achenes with and without dissection.

Pathogenicity during storage was determined by exposing full achenes to spores and mycelia of three of the most prevalent field fungi and two of the most prevalent storage fungi. Each fungus was grown for 10 days on steam-sterilized oat seeds (*Avena sativa* L.). About 3,000 full achenes were selected and their MC adjusted to 15% by adding sterile distilled water. About 250 achenes were combined with about 30 inoculated oat seeds or with sterile oat seeds (controls). Half of each mixture was placed in a 150-ml metal can in which holes had been punched to give sharp metal edges on the inside; the other half was placed in a similar can without holes. The two cans were then shaken to inoculate the achenes by thoroughly mixing them with the oat seeds, and, in the case of the can with holes, to scarify the achenes. In this way, achenes were inoculated with each test fungus and with the control, with and without scarification. The achenes were then stored at 30 ± 1 C and 84-86% RH for 10 and 35 days. Germinability and fungi associated with these four groups of achenes were determined as in the survey study.

RESULTS AND DISCUSSION.—Only the major conclusions from these studies are presented. Details are reported in the thesis from which this paper is derived (4).

Survey.—Germinability of the seeds varied from 17 to 72% for mixed achenes and from 81 to 97% for full achenes. This variation among mixed achenes from different locations is similar to the 16 to 68% observed in a recent provenance test of sycamore achenes (18). The difference in percentage germinability between mixed and full achenes indicates the frequency with which achenes without seeds are produced in certain provenances. The MC of the achenes varied from 5.8 to 11.6%, depending on the location and year. Variation in germinability was not correlated with the MC of the achenes or the location, physiographic region, or the year of collection.

No appreciable differences were observed in the total number of fungi isolated on the two media. Thus, only the data obtained with PSM are presented to indicate the prevalence of the predominant genera of fungi isolated (Tables 1, 2).

More than 10,000 fungi from 26 genera were iso-

TABLE 1. Relative prevalence of the predominant genera of fungi isolated on potato-salt medium from sycamore achenes collected in 1967 at nine locations in the major physiographic regions of the southern United States

Genera of fungi[a]	Two Mountain locations		Four Piedmont locations		Three Coastal Plain locations	
	Pericarps	Seeds	Pericarps	Seeds	Pericarps	Seeds
	% pericarps or seeds yielding fungi in each genus					
Alternaria	61	5	56	5	44	6
Aureobasidium	25	1	21	0	6	0
Cladosporium	69	0	35	0	19	0
Epicoccum	15	1	12	0	6	0
Fusarium	8	0	7	0	4	0
Pestalotia	2	0	11	0	15	0
Peyronellaea	3	0	34	4	2	0
Phoma	9	2	16	4	13	4
Phomopsis	22	17	6	2	25	9
Xylaria	1	0	1	0	9	0
Total no.						
Genera/location	8-12	1-5	8-15	2-6	8-11	2-4
Isolates/location	232-236	8-43	111-268	2-25	95-188	5-23

[a] Other fungi isolated included members of *Aspergillus, Chaetomium, Coniothyrium, Diplodia,* and *Sordaria.*

TABLE 2. Relative prevalence of the fungi isolated on the potato-salt medium from sycamore achenes collected in 1967, 1968, and 1969 at four locations in North Carolina

Genera of fungi[a]	1967		1968		1969	
	Pericarps	Seeds	Pericarps	Seeds	Pericarps	Seeds
	% pericarps or seeds yielding fungi in each genus					
Alternaria	67	8	81	9	62	5
Aureobasidium	25	1	30	1	19	0
Cladosporium	30	0	44	0	32	0
Epicoccum	19	1	29	1	19	1
Fusarium	9	0	12	0	10	0
Pestalotia	1	0	2	0	1	0
Peyronellaea	35	5	47	4	29	4
Phoma	21	7	21	7	15	3
Phomopsis	6	2	11	3	17	7
Xylaria	3	0	3	0	1	0
Total no.						
Genera/location	8-15	4-6	10-12	3-6	8-12	3-4
Isolates/location	188-268	17-25	247-309	20-30	128-256	12-37

[a] Other fungi isolated included members of *Coniothyrium, Diplodia, Penicillium, Pestalotia,* and *Sordaria.*

TABLE 3. Species identification of fungi isolated during the survey and storage studies

Alternaria sp. I	*Fusarium roseum* Lk. emend. Snyd. & Hans.
Alternaria sp. II[a]	*Fusarium moniliforme* Sheldon
Aspergillus amstelodami (Mang.) Thom & Church	*Gloesporium* sp.
Aspergillus niger Van Tieghem	*Helminthosporium spiciferum* (Bainier) Nicot
Aspergillus ochraceous Wilhelm	*Nigrospora* sp.
Aspergillus repens (DeBary)[a]	*Nodulisporium hinnuleum* Smith[a]
Aspergillus ruber (K. S. & B.)[a] Thom & Church	*Penicillium brevi-compactum* series
Aureobasidium pullulans (de Bary) Arn	*Penicillium* sp.
Bipolaris sp.	*Pestalotia* sp. I
Calcarisporium sp.	*Pestalotia* sp. II
Chaetomium cochliodes Palliser	*Pestalotia* sp. III
Cladosporium cladosporoides (Fres.) nov. comb.	*Peyronellaea* sp.
Coniothyrium sp.	*Phoma* sp. I
Curvularia intermedia Boedijn	*Phoma* sp. II
Cytospora sp.	*Phomopsis* sp.
Diplodia sp.	*Sordaria fimicola* (Rob.) Ces. & DeNot.
Diplodina sp.	*Stemphyllium* sp.
Epicoccum nigrum Link[a]	*Trichoderma konigii* Oud.
	Xylaria sp. I
	Xylaria sp. II

[a] These fungi were used in the test for pathogenicity during storage.

lated from the achenes (Table 3). These fungi included members of three genera of Ascomycetes and 21 genera of Fungi Imperfecti. The total number of fungi as well as the prevalence of individual genera varied markedly with the physiographic region (Table 1), location (Fig. 1) and year of collection (Table 2, Fig. 1). The abundance and diversity of these fungi is probably a consequence of the long time (6-8 months) sycamore fruit balls were exposed on the trees before the achenes were collected.

Fungi isolated from the achenes most frequently included members of the genera *Alternaria, Aureobasidium, Cladosporium, Epicoccum, Fusarium, Pestalotia, Peyronellaea, Phoma, Phomopsis* and *Xylaria* (Tables 1, 2). The average achene yielded isolates of more than two fungi. Pericarps invariably yielded more fungi than seeds.

Many of these fungi also have been reported from seeds of other forest trees (5, 9, 10, 11, 16, 17). Similarly, the variability in prevalence of fungi with location is consistent with reports of fungi associated with seeds of oak (13), six other hardwoods (5), and various pines (9). The predominant fungi isolated from sycamore achenes are similar to the "field fungi" reported for seeds of wheat, corn, and barley (3).

Storage study.—During storage, achenes from both Raleigh (city) and Tarboro (forest) showed similar patterns of change in MC, germinability, and fungi isolated. The MC of the achenes increased rapidly during the first 2 months in storage, but much more slowly thereafter (Fig. 2). The achenes failed to reach equilibrium MC even after storage for 7 months. This slightly increasing MC was probably due to water accumulating from either (i) respiration of the fungi associated with the achenes; (ii) respiration of the living cells of the achenes; (iii) differential rates of moisture adsorption and desorption in the imperfectly controlled temperature of the incubators or a combination of these factors: Probably (i) and (ii) are involved at 20 and 30 C, but only (iii) at 2 C.

During storage at 2 C, seed germinability did not change, irrespective of the time or RH (Fig. 3). At 20 and 30 C, however, germinability decreased rapidly and was related directly to the temperature-time and RH of storage. Very few or no seeds germinated after 4 or 7 months at 30 C (Fig. 3). These substantial losses in germinability were associated with small changes in MC (Fig. 2). After 2 months at 30 C and at 79-82 and 84-88% RH, for example, germinability

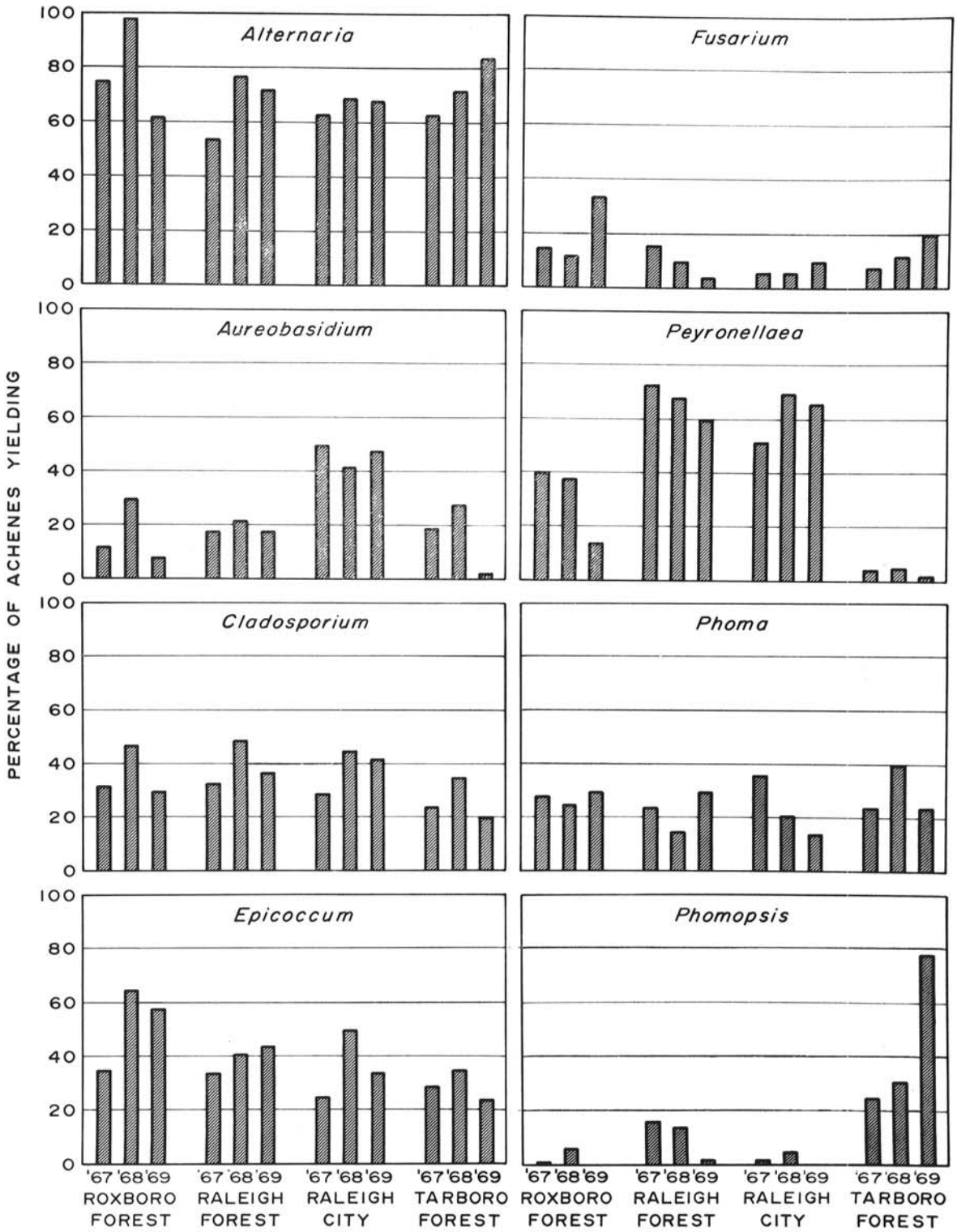

Fig. 1. Relative prevalence of fungi associated with sycamore achenes collected in 1967, 1968, and 1969 at four locations in North Carolina.

was reduced to 23% at 13.0% MC and to only 3% at 13.9% MC. This rapid loss in germinability and the differences in rate of loss associated with relatively small differences in MC agree with similar changes observed in cereal grains (2).

During storage at 2 C, only small changes were observed in the prevalence of the various fungi associated with the achenes. At 20 and 30 C, however, marked changes in fungal flora were observed. Field fungi including members of the genera *Alternaria, Aureobasidium, Cladosporium, Epicoccum, Fusarium, Peyronellaea,* and *Phoma* decreased rapidly in prevalence with increasing time in storage (Fig. 4). The data presented in Fig. 4 for *Alternaria* also represent the changes observed in prevalence of the other genera. Storage fungi including *Aspergillus ruber, A. amstelodami,* and *A. repens* were not isolated from the achenes at the beginning of the storage study. Their prevalence in both pericarps and seeds increased markedly with time in storage (Fig. 5). Only data for *A. ruber* is presented because the changes for *A. repens* and *A. amstelodami* were similar.

These changes in prevalence of field and storage fungi isolated from sycamore achenes are similar to

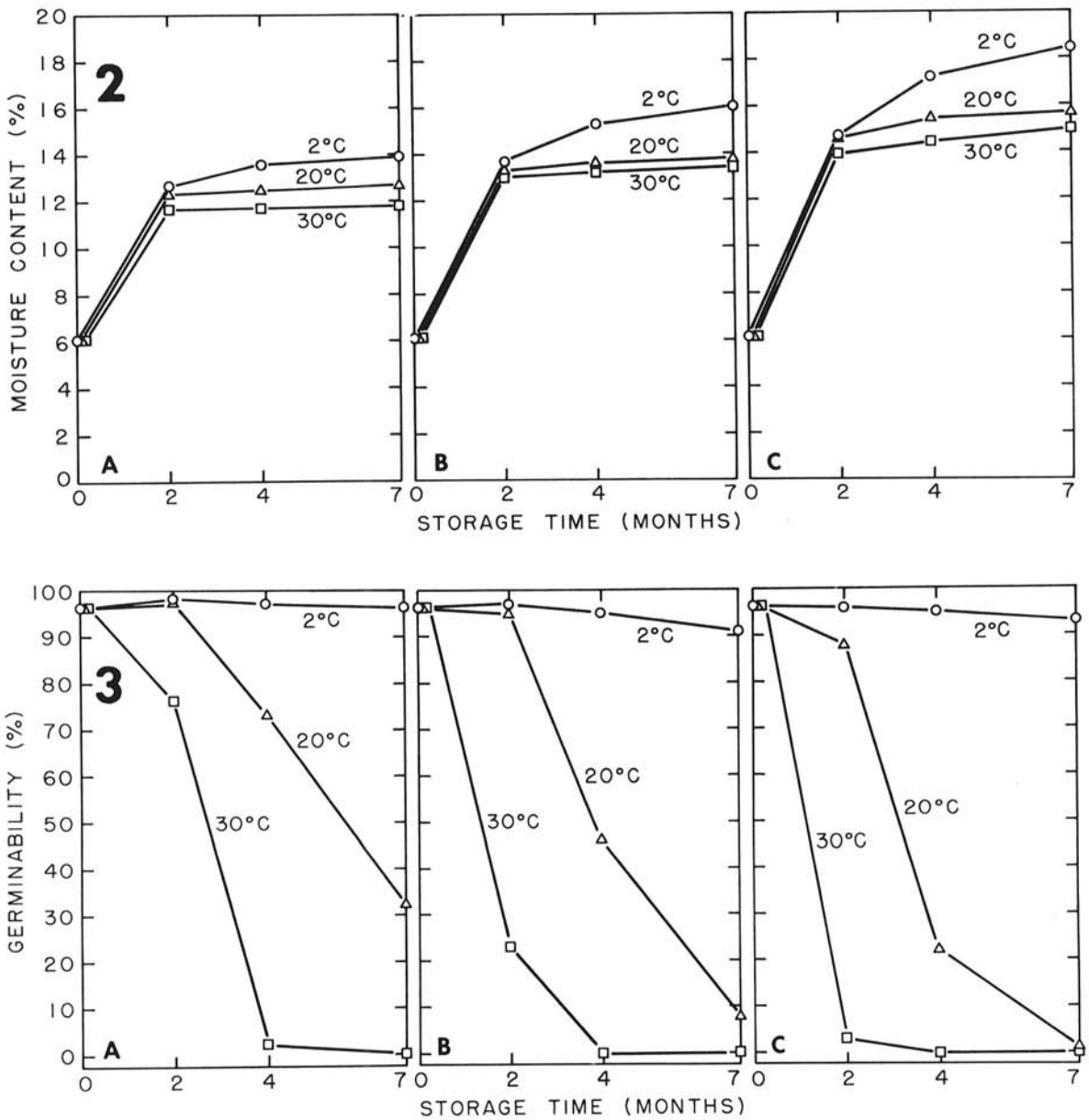

Fig. 2-3. **2)** Moisture content of sycamore achenes collected in the city of Raleigh, N.C., and stored at 2, 20, and 30 C at (A) 74-76%; (B) 79-82%; and (C) 84-88% relative humidity. **3)** Germinability of sycamore achenes collected in the city of Raleigh, N.C., stored at 2, 20, and 30 C at (A) 74-76%; (B) 79-82%; and (C) 84-88% relative humidity.

changes observed in the prevalence of these same genera of fungi with the cereal grains during storage (Fig. 4, 5) (3). All field fungi decreased in prevalence with time in storage. The rate of decrease was related directly to the temperature and RH of storage. After storage, most of the field fungi could not be isolated at all, or could be isolated from only a few intact achenes or pericarps. No field fungi could be isolated from the seeds after storage for 4-7 months at 20 or 30 C, irrespective of RH.

All storage fungi increased in prevalence with time in storage. The rate of increase also was related directly to the temperature and RH. Growth and development of storage fungi, particularly of *Aspergillus* spp., were accompanied by the formation of fruiting bodies, i.e., conidiophores and cleistothecia (Fig. 6).

The rapid reduction in germinability during storage at 20 and 30 C (Fig. 3) was associated with a marked decrease in prevalence of field fungi (Fig. 4) and an equally striking increase in prevalence of storage fungi (Fig. 5). High germinability despite the prevalence of many field fungi in both pericarps and seeds prior to storage, and the reduction in prevalence of most field fungi with time in storage, suggest that the field fungi probably did not exert a major influence on germinability when achenes were stored. The relationship between loss in germinability and percentage of dissected full achenes yielding species of *Aspergillus* is shown in Fig. 3 and 5. This relationship, coupled with the demonstrated capacity of several species of *Aspergillus* to reduce the germinability of cereal grains and other dry seeds in storage (3), suggests that these fungi may have caused loss in germinability when achenes were stored at 20-30 C and approx 12-16% MC for 2-4 months or longer. Possibly death of embryos may have resulted from exhaustion of stored food reserves at the high MC and temperature of storage, and storage fungi may have invaded the dead or weakened seeds. This possibility is consistent with the observation that the percentage of achenes that did not germinate was sometimes substantially higher than the percentage of seeds yielding one or more species of *Aspergillus* (96%

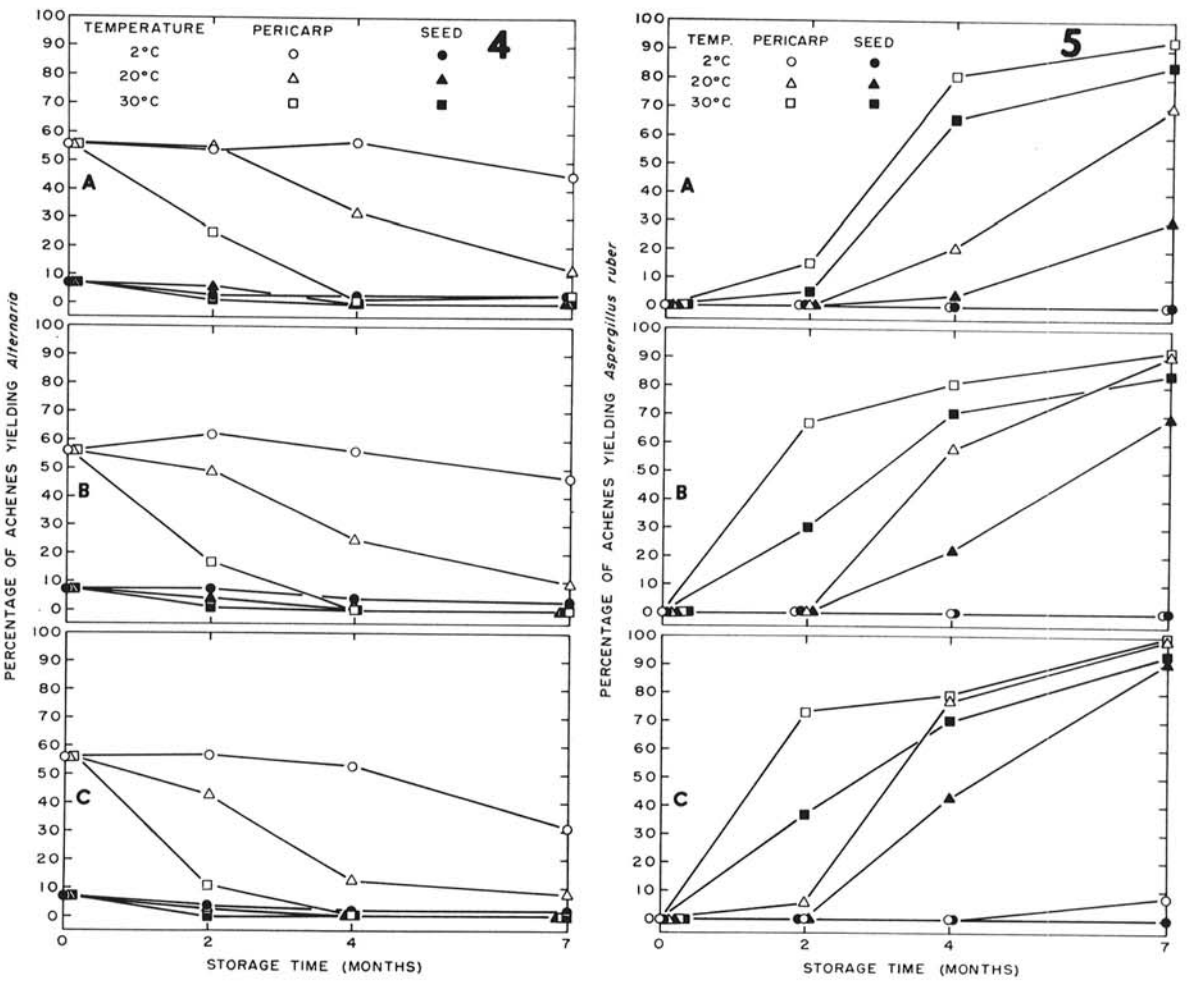

Fig. 4-5. **4)** Changes in prevalence of *Alternaria* during storage of sycamore achenes at (A) 74-76%; (B) 79-82%; and (C) 84-88% relative humidity. **5)** Changes in prevalence of *Aspergillus ruber* during storage at (A) 74-76%; (B) 79-82%; and (C) 84-88% relative humidity.

TABLE 4. Changes in germinability and fungus flora isolated from pericarps (P) and seeds (S) during storage of nonscarified sycamore achenes after inoculation with the most prevalent field and storage fungi

Inoculation treatment	Time in storage, days	Germinability, %	*Alternaria* spp.[a]		*Aspergillus repens*[b]		*Aspergillus* ruber[b]		*Epicoccum nigrum*[a]		*Nodulisporium hinnuleum*[a]		Other fungi[c]	
			P	S	P	S	P	S	P	S	P	S	P	S
Noninoculated control	0	96	72	20	0	0	0	0	30	4	0	0	96	18
	10	94	55	15	5	0	10	0	10	0	0	0	55	10
	35	62	35	5	20	5	75	35	5	0	0	0	30	5
Field fungi	10	95	100	25	0	0	10	0	0	0	0	0	20	0
Alternaria sp. I	35	66	80	10	20	10	70	25	0	0	0	0	10	0
Epicoccum	10	96	30	5	5	0	10	0	60	10	0	0	20	5
nigrum	35	54	10	0	10	0	90	30	20	0	0	0	5	0
Nodulisporium	10	92	40	5	0	0	20	5	5	0	50	0	10	0
hinnuleum	35	58	20	0	5	0	90	35	0	0	15	0	0	0
Storage fungi														
Aspergillus	10	91	20	5	45	10	0	0	0	0	0	0	5	5
repens	35	44	5	0	100	40	0	0	0	0	0	0	0	0
Aspergillus	10	90	15	5	0	0	50	10	0	0	0	0	20	0
ruber	35	42	5	0	0	0	100	45	0	0	0	0	0	0

[a] Isolated on rose-bengal medium.
[b] Isolated on potato-salt medium.
[c] Other fungi isolated from pericarps included *Aspergillus niger, Aureobasidium pullulans, Cladosporium cladosporoides, Penicillium* spp., *Peyronellaea* sp., *Phoma* spp., *Phomopsis* sp., *Xylaria* sp., and unknown. Other fungi isolated from seeds included only *Phoma* spp. and *Phomopsis* sp.

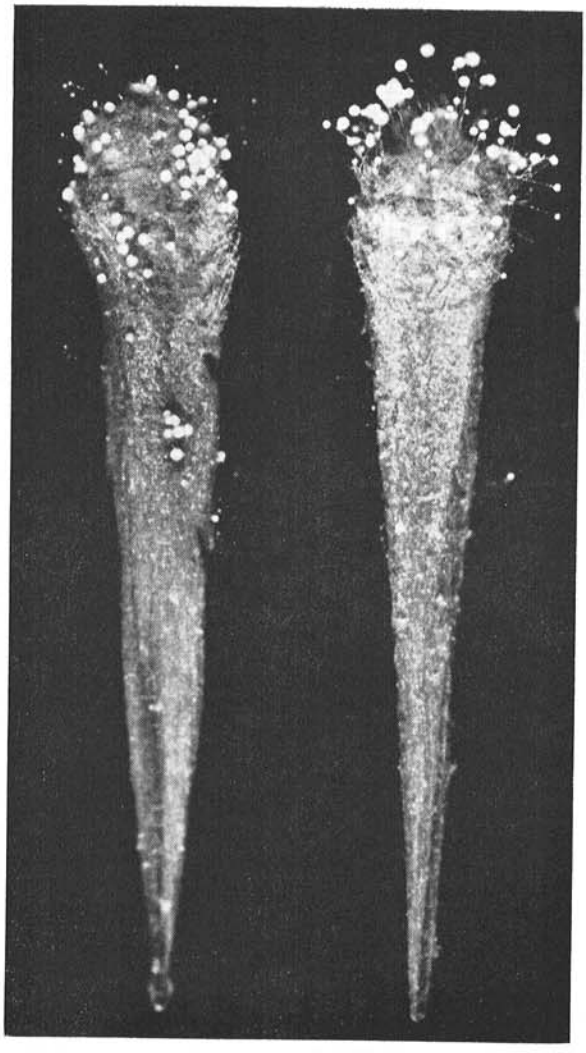

Fig. 6. Sycamore achenes showing cleistothecia and conidiophores of *Aspergillus* spp. that developed during storage.

loss in germinability with 72% of the pericarps infected, but only 37% of the seeds infected by *A. ruber* after 2 months at 30 C and 84-88% RH) (Fig. 3 & 5).

Data on cereal grains suggested that the MC achieved in equilibrium with 75% RH would be sufficiently low to avoid rapid loss in germinability (3). The critical MC, below which losses in germinability should not occur, and *Aspergillus* spp. should not infect sycamore achenes is below 11.7% (Fig. 3). This is somewhat below the critical MC for starchy seeds of other plants (3).

Pathogenicity test.—In the test of pathogenicity during storage, only negligible differences in germinability or fungal flora were observed between scarified and nonscarified achenes. Thus, only the results for nonscarified achenes are presented (Table 4).

Germination was normal and showed no significant decrease when inoculated achenes were stored for 10 days. After 35 days, however, germinability decreased markedly; the noninoculated controls decreased to 62%, while those inoculated with *Aspergillus repens* and *A. ruber* decreased to 44 and 42%, respectively.

Each of the five fungi used for inoculation was reisolated after 10 and 35 days in storage although the percentage of achenes yielding each particular fungus varied considerably depending on the fungus and the storage time (Table 4). In the survey and storage studies, all achenes, inoculated or not, yielded a diverse fungal flora; also, pericarps yielded higher percentages of all fungi than did seeds. Invasion of the achenes by the test fungi is demonstrated by the fact that the highest percentages of pericarps and seeds yielding each test fungus were observed with the achenes inoculated with that particular fungus. Invasion by the two *Aspergillus* spp. is demonstrated by the higher percentages of pericarps and seeds yielding each of these fungi after 35 days than after 10 days. In the storage study, the two field fungi tested, *Alternaria* sp. and *Epicoccum nigrum,* decreased in prevalence, while two of the storage fungi, *Aspergillus repens* and *Aspergillus ruber,* increased in prevalence with time in storage. *Nodulisporium* sp. (*hinnuleum*) was included in this study because it increased during the storage study. However, it decreased with time in this pathogenicity test. This suggests that *N. hinnuleum* probably is not a typical storage fungus. With this minor exception, the results of this study confirm the distinctive characteristics of field and storage fungi in relation to sycamore achenes during storage.

The greater reduction in germinability after inoculation with *Aspergillus repens* and *A. ruber,* compared to those noninoculated or those inoculated with the other test fungi, indicates that these two *Aspergillus* spp. can cause injury during storage. In the storage study, the cause of the decrease in germinability of noninoculated achenes cannot be identified unequivocally; either the physical conditions of storage (30 C and 82-86% RH for 35 days in this case) or the organisms that developed under these conditions (or a combination of both) could have caused the reduction in germinability.

CONCLUSIONS.—Our results show that (i) sycamore achenes have an abundant and diverse fungal flora; (ii) changes in this flora during storage are similar to those reported for cereal grains, except that the min MC for fungal growth in stored sycamore achenes is lower than that required for starchy seeds of other plants; (iii) sycamore achenes containing as much as 19% moisture can be stored at 2 C up to 7 months without loss in germinability, but achenes with as little as 13% moisture cannot be stored at 20 or 30 C even for 2 months; and (iv) *Aspergillus ruber* and *A. repens* are probably capable of reducing germinability of sycamore achenes during storage.

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