FUNGI ASSOCIATED WITH THE PISTILLATE FLOWERS OF WHITE OAK (*Quercus alba* L.) AND THEIR EFFECT ON POLLEN GERMINATION

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF TABLES (Cont'd)

INTRODUCTION

White oak (Quercus alba L.) is a prominent member of the deciduous forest of the eastern United States. It occurs on a wide variety of sites and soil types and is indigenous to the major climatic regions of the East (USDA, 1965). Prior to the introduction of the chestnut blight, white oak commonly occurred in association with American chestnut (Castanea dentata). The severe reduction of the chestnut population by the blight has led to a substantial increase in the white oak population (Keever, 1973).

White oak is of considerable importance in timber production and wildlife management. It is a valuable commercial species and produces high quality lumber. Lumber from white oak is used in the production of furniture, flooring, veneer, railroad ties, cooperage and a variety of other products (Panshin and deZeeuw, 1970). The wood is hard, durable, impermeable to liquids and can be sanded to a smooth finish. In addition to providing the raw materials for wood products, white oak is an important source of food for major eastern wildlife species.

The form and quality and hence the value of white oak trees has steadily degenerated over the last century (Liming and Johnston, 1944). Highgrading followed by coppice regeneration has produced stands of trees with many poor quality stems. As a consequence, current stands of white oak are not producing high quality products.

In order to correct the effects of highgrading, tree improvement programs and better methods for reforesting cutover areas with improved growing stock must be developed. Artificial regeneration of hardwood

stands by planting or seeding is difficult since control of the natural regeneration, primarily sprouts, is a problem. White oak seedlings grow very slowly their first few years and although they tolerate shade, the competition from native vegetation hinders their growth and is undesirable. Planting oak seedlings as a means of reproducing a stand requires a substantial initial expenditure. However, efforts in this area have been limited and improvements in regeneration methods will follow as more research is performed (Russel, 1971).

Tree improvement programs for white oak have yet to be established on a large scale. One obstacle to the development of improvement programs is erratic seed production. Good seed years in white oak occur infrequently, and the time span between them may reach four to ten years (Harlow and Harrar, 1969). Since the efficiency of tree improvement programs depends on a yearly seed set, the lack of a regular seed crop has discouraged breeding.

Attempts have been made to elicit a cause and effect relationship between acorn production and environmental factors. However, with the exception of springtime air temperatures (Sharp and Sprague, 1967), no relationship between changes in an environmental factor and success of the acorn crop has been reported in the literature.

An understanding of the causal factors controlling acorn production in white oak will be helpful in developing a predictive model to estimate the size of the yearly acorn crop. In addition, manipulation of one or more controlling factors may be possible so that yearly acorn crops are produced.

The intent of this research project is to investigate the occurrence of fungi on white oak flowers and to measure the effect of commonly occurring fungi on pollen germination. The presence or absence of these organisms on the pistillate flowers during the period of pollination may significantly influence the acorn crop. If this is true, manipulation of the level of occurrence of the fungi, either through inoculation of the flowers with spores or application of fungicides, could improve the consistency of acorn production.

LITERATURE REVIEW

The mechanism of reproduction in Quercus was first investigated in detail by Conrad (1900). Working with Quercus velutina, he described ovule development, microsporogenesis and embryogenesis up to the early embryo stage. Later studies on the development of the embryo sac were reported by Langdon (1939) on Q. rubra and Hjelmqvist (1953) on Q. robur. Microsporogenesis and embryogenesis in Q. alba, Q. coccinea and Q. illicifolia were studied by Stairs (1964).

A complete outline of the reproductive cycle of Q. alba was presented by Turkel et al. (1955). They described the development of the pistillate and staminate flowers from the time of their initiation during vegetative growth of the year prior to their appearance up until the time of fertilization. An abscission layer was reported to develop at the base of the ovary as early as the time of pollination. This was believed to cause the early release of the immature acorns.

The abortion of ovules in Q. gambelii, Q. alba and Q. velutina was studied by Mogenson (1975). He found four different kinds of aborted ovules, the most common type being those that develop normally but are not fertilized. He postulated that the first ovule fertilized in an ovary suppresses the development of those remaining.

An examination of the stages of staminate flowering and pollen dispersal in white oak was made by Sharp and Chisman (1961). They reported the occurrence of a heavy crop of staminate flowers each year on most of the trees in their study. The time of flowering varied among the

trees and the abundance of catkins produced on individual trees was consistent from year to year.

Catkins first appeared in the topmost branches of the trees and were more abundant there than in the lower branches. Relative humidity and air temperatures were found to be the controlling factors for pollen release. Anthesis occured over a period of three days although rain or cold temperatures prolonged the time of pollen release.

Pistillate flowering and acorn yields in white oak were studied by Sharp and Sprague (1967). They examined the effect of weather on flower development and acorn production. Wind, relative humidity and vapor pressure deficits did not significantly influence acorn yields. Spring-time air temperatures did affect acorn production. Good acorn crops occurred in years when a warm 10-day period in late April was followed by a prolonged period of cool temperatures in early May. Sharp and Sprague (1967) believe that the early warm period may favor the development of viable pollen and the subsequent cool period enhance ovary development and fertilization.

Acorn production on individual trees occurred consistently each good seed year while other trees generally produced a poor crop or none at all. Sharp and Sprague suggested acorn production is under a significant degree of genetic control and thus can be improved by breeding.

The pistillate flowers of white oak occur at the end of a short stalk in the leaf axils of the current year's growth, generally in clusters of two to five. Each flower is bulbous in appearance with a short yellow bowl-shaped style. As the flower matures, the style separates, usually into three segments, each of which elongates and becomes reflexed (Sharp

and Sprague, 1967). The flowers occur in high concentration in the upper crown area and in low concentration in the lower crown area.

The development of breeding techniques for white oak was investigated by Ledig, Beland and Fryer (1971). Methods of pollen storage and controlled pollination were studied. Pollen was stored using different combinations of freeze drying with or without prefreezing and storage under vacuum or nitrogen gas. Pollen viability was measured before and after storage as a percent germination on an agar medium containing sucrose and boric acid. The most effective storage method was prefreezing followed by freeze drying for ten minutes with storage under nitrogen gas. Pollen stored for one year in this manner was still capable of initiating seed set.

MATERIALS AND METHODS

PART I. ISOLATION OF THE FUNGI - 1976

Pistillate flowers were collected from four mature white oaks in the spring of 1976. Two of the trees were located in the Piedmont physiographic zone at the Reynolds Homestead Research Center near Critz, Virginia. The remaining two trees were located in the Ridge and Valley geomorphic province at Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The area surrounding the Critz trees is covered by an oak-hickory forest type with scattered stands of Virginia pine. The two trees chosen were situated at the perimeter of a Virginia pine stand and were in the dominant crown class. They were selected for this study based on their large crown size, potential for seed production and accessibility.

The two trees chosen at Blacksburg were also selected on the basis of their crown size, potential for seed production and accessibility. Both trees possessed full, well-developed crowns that were exposed to direct sunlight.

Flower Collection at Critz

The two trees sampled at Critz produced an abundance of pistillate flowers in 1976. Most flowers appeared completely developed a day or two prior to pollen release. Towers were built adjacent to each tree to permit access to all levels of the tree crown. Flower collection was initiated on April 19, 1976. At this time staminate catkins were pendant and anthesis appeared likely in 24 to 48 hours. Flowers were collected

during two time periods--0830 to 1030 and 1400 to 1600 EDT. Samples were taken from three levels of the tree crown to map the vertical distribution of the fungi throughout each tree. During each collection period five culture plates containing a general culture medium (potato dextrose agar) were exposed to obtain a sample of the airspora at collection time.

Flowers were collected using forceps sterilized by immersion in 95% ethanol and flamed over an alcohol lamp. A flower cluster was pinched off at the base of its stalk with the forceps and placed in a sterile collection vial. The forceps were sterilized after each flower cluster was collected to prevent transferring fungi from one cluster to the next. Thirty flowers were collected per tree during each collection period.

Pollen release on Tree 1 began April 19 and was completed by April 21. Flowers were collected during this time and for three days afterward. Three hundred flowers were obtained from Tree 1. Anthesis on Tree 2 began on April 20 and ended April 22. Two hundred and forty flowers were collected from Tree 2. The schedule followed in collecting the flowers is presented in Table 1.

Flower Collection at Blacksburg

Flowering in the white oaks occurred one week later at Blacksburg than at Critz. Flowers were collected from branches cut from the lower crown with a pole pruner. All branches were caught before striking the ground to prevent contamination of the flowers by soil borne fungi. The branches were taken to the laboratory and the flowers collected. Since

Table 1. Flower collection schedule for Critz (1976).

	Tree 1		Tree 2	
Date	0830-1030[1]	1400-1600	0830-1030	1400-1600
4/19/76	30[2]	30	30	30
4/20/76	30	30	--	--
4/21/76	30	30	30	30
4/23/76	30	30	30	30
4/24/76	30	30	30	30
	Total = 300		Total = 240	

[1] Time period (EDT).

[2] Number of flowers collected.

only the lower branches of the tree were within reach of the pruner, no flowers were collected from the upper crown area.

At Blacksburg, flower collection was begun April 25 and completed May 5 for both trees. The catkins were pendant on April 26, but air temperatures dropped below 15 C for several days and delayed anthesis until May 3. Flower collections were scheduled over a 10-day period to include the time of anthesis. The schedule followed in collecting at Blacksburg is presented in Table 2.

Culturing the Fungi

Flowers were placed onto agar media as soon after collection as possible. The flowers were removed from the vials and a single flower excised from each cluster. Each flower was sectioned longitudinally and placed on a culture plate. The purpose of sectioning was to expose fungi, if present within the flower, to the media.

All cultures were incubated at 27 C until colonies of fungi had completely covered the growing surface. They were then removed from the incubator and placed under fluorescent lights for a week to induce sporulation (Tuite, 1969). Following sporulation, cultures were placed in storage at 4 C until each was examined.

Three culture media were used to isolate fungi. They were potato-dextrose agar (PDA), potato-dextrose agar with chloramphenicol (CPDA), and potato-dextrose agar with chloramphenicol and BOTRAN™ (BCPDA). PDA is a general nonselective culture medium (Tuite, 1969) and was chosen since it provides the proper growing conditions for a wide variety of fungi. CPDA is a semiselective medium inhibiting bacterial growth

Table 2. Flower collection schedule followed at Blacksburg (1976).

	Tree 1		Tree 2	
Date	0830-1030[1/]	1400-1600	0830-1030	1400-1600
4/25/76	--	30[2/]	--	30
4/26/76	30	30	30	30
4/27/76	30	30	30	30
4/28/76	30	--	30	--
4/30/76	30	--	30	--
5/1/76	--	30	--	30
5/3/76	30	--	30	--
5/5/76	30	--	30	--
	Total = 300		Total = 300	

[1/] Time Period (EDT).

[2/] Number of flowers collected.

(Stipes, 1973). Chloramphenicol is a bacterial antibiotic which suppresses bacterial growth in low concentrations (0.04%) but does not affect the growth of most fungi (Tuite, 1969).

BCPDA is a semiselective medium suppressing the growth of bacteria and Phycomycetes. Phycomycetes are rapidly growing fungi and can mask the presence of other fungi in a culture. BOTRAN™ is a commercial fungicide which will selectively inhibit the growth of Phycomycetes in low concentration (0.0004%). This medium and the CPDA were used to preclude the masking of slow growing fungal colonies by bacteria or Phycomycetes. Formulas for the media used are presented in Table 3.

The fungi growing from the oak flowers were identified using Barnett and Hunter's Illustrated Genera of Imperfect Fungi (Barnett and Hunter, 1972). Wet mounts were made of the various colonies of fungi occurring in the culture plates. The fungi were then identified according to the structure of their fruiting bodies. Several colonies of fungi were sterile and thus could not be identified. Unidentified fungi were categorized according to colony color and morphology and given a descriptive label (see Appendix).

Table 3. Culture media formulas.

I) Potato Dextrose Agar (PDA)

potatoes	250 grams
dextrose	10 grams
agar	18 grams
microelements[1/]	2 ml
distilled H_2O to 1 liter	

II) PDA plus Chloramphenicol (CPDA)

After autoclaving one liter of PDA, add 400 milligrams of Chloramphenicol dissolved in 10 ml of 95% ethanol.

III) PDA plus Chloramphenicol and BOTRAN™ (BCPDA)

Prepare one liter of CPDA and add 2.7 milligrams of BOTRAN™ dissolved in 2.7 ml of acetone.

[1/] Fe (0.01%), Mn (0.005%), Zn (0.01%)

PART II. ISOLATION OF THE FUNGI - 1977

Flowers were collected from four trees at Blacksburg in 1977, the two trees sampled in 1976 (referred to here as Trees 1 and 2) and two other mature white oaks (referred to here as Trees 3 and 4). An airspora analysis was also performed. No flowers were collected in 1977 at Critz due to a widespread failure of the flower crop.

Flower collection was initiated on April 20 and completed April 26. Flowers matured first on Tree 3 and last on Trees 1 and 2. Anthesis occurred April 22 on Tree 3, April 23 on Tree 4, but was delayed until April 30 on Trees 1 and 2 due to a drop in air temperatures below 15 C starting April 24 and lasting several days. This was similar to the delay in anthesis caused by low temperatures the previous year. The collection schedule followed is presented in Table 4.

A smaller number of flowers (120) was collected from each tree in 1977. Sixty flowers per tree were cultured immediately after collection. Of the remaining flowers, thirty were surface sterilized before culturing to determine whether the fungi occur predominantly on the surface or in the interior of the flower. The surface sterilization was accomplished by immersing the flowers into a solution of 1.0% sodium hypochlorite for two minutes followed by rinsing in sterile distilled water for two minutes (Tuite, 1969).

The remaining thirty flowers from each tree were stored in collection vials at 4 C for four weeks prior to culturing to determine whether storage decreased fungal viability. After four weeks, the flowers were

Table 4. Flower collection schedule followed at Blacksburg (1977).

Date	Tree 1	Tree 2	Tree 3	Tree 4
4/20/77	--	--	60[1/]	--
4/22/77	--	60	60	--
4/23/77	60	--	--	60
4/24/77	--	60	--	--
4/26/77	60	--	--	60
Totals	120	120	120	120

[1/] Number of flowers collected.

removed from storage and cultured on agar. A summary of the treatments and number of flowers collected for each is presented in Table 5.

The culture medium used for the 1977 isolations was CPDA. Results of the previous year indicated that the type of culture media had no significant effect on the isolation of fungi. Also, the problems anticipated from bacteria and Phycomycetes did not arise. Therefore, each of the three media was acceptable for use. CPDA was chosen to keep undesirable bacterial growth to a minimum for easier fungi identification.

For the airspora analysis, ten plates of PDA were left exposed in the vicinity of the sample trees from 0830 to 1030 EDT for seven consecutive days starting April 19.

Table 5. Outline of the treatments performed with the flowers collected at Blacksburg in 1977.

	Treatments[1/]		
	NT	SS	ST
Tree 1	60	30	30
Tree 2	60	30	30
Tree 3	60	30	30
Tree 4	60	30	30
Totals =	240	120	120

[1/] NT = No treatment.

SS = Surface sterilized.

ST = Storage at 4 C for four weeks followed by culturing.

PART III. TESTING THE INFLUENCE OF FUNGI ON POLLEN GERMINATION

Four interaction tests were designed to determine whether pollen germination is significantly increased or decreased by the fungi. Using the test procedures outlined in the following paragraphs, pollen was germinated in the presence of fungal spores or hyphae and the germination success compared to that of pollen germinated without fungi.

Pollen was obtained from two white oaks by collecting catkins and placing them on sheets of paper in a dry room. After 24 hours, anthesis occurred. The pollen was recovered from the catkins and cleaned by sifting the catkins on a 60-mesh screen and then an 80-mesh screen. Germination tests indicated that pollen from each tree was equally viable (45 and 51 percent germination). The pollen from each tree was combined and the resultant mixture used for all tests. A total of four interaction tests were performed.

For the first test, a suspension of pollen grains and fungal spores was prepared in sterile distilled water. The suspension was spotted onto an agar medium favorable to both and then incubated at room temperature for 12 hours in the dark (see Table 6 for medium formula).

Significant results were not obtained from Test 1, however, since inadequate pollen germination was observed in the controls as well as with the spore suspensions (0 to 9 percent). This test was discontinued.

Two additional tests were designed whereby a suspension of pollen grains in sterile distilled water was spotted onto an agar medium (Table 6) containing either a lawn of fungal mycelium or exogenous substances released by the fungi while growing in a liquid medium. Low

Table 6. Formulas for interaction test media.

Medium for Tests 1-3

sucrose	50 grams
boric acid	0.1 gram
agar	18 grams
microelements	2 milliliters
distilled H_2O	1 liter

Medium for Test 4

sucrose	50 grams
boric acid	0.1 grams
yeast extract	1 gram
agar	1 gram
microelements	2 milliliters
distilled H_2O	1 liter

pollen germination was also experienced in these tests and they were discontinued.

Successful pollen germination was obtained in a fourth test in which pollen grains and fungi spores were combined in a liquid medium and a drop of this mixture cultured in a Van Tieghem cell. Pollen germination under these conditions averaged 40 percent. This was considered sufficient for further experimentation.

The liquid medium used for Test 4 contained 5% sucrose, 1% agar, 0.1% yeast extract, 0.01% boric acid and microelements (Table 6). Sucrose and boric acid provide the necessary elements for pollen germination. The yeast extract, sucrose and microelements provide the materials necessary for the growth of fungi. Agar was added to increase the viscosity of the liquid to assist in obtaining a hanging drop.

One hundred and twenty Van Tieghem cells were prepared. The cells were made by cementing 2 cm sections of 2.0 cm O.D. glass tubing to a glass microscope slide with an epoxy glue. All cells were sterilized in a forced air oven at 170 C for three hours prior to each use. Glass cover slips were used and were also oven sterilized. All other materials for Test 4 were sterilized by autoclaving for 30 minutes at 121 C and 15 PSI.

Spore suspensions for each fungus were prepared by scraping the mycelia from pure sporulating cultures into a 250 ml erlenmeyer flask containing 50 ml of liquid media and a stirring bar. The mycelia were then agitated on a magnetic stirrer for 15 minutes at low speed to loosen the spores. The resultant suspension of spores and mycelia was strained through a double layer of sterile cheesecloth, thus removing

the mycelia. The resultant suspension of fungal spores was used for the interaction tests.

Once the initial suspension of a fungus was prepared, two additional suspensions were made which were 1:9 and 1:99 dilutions of the first. A fourth flask containing only sterile media was prepared as a control.

Pollen was added to each flask at a concentration of 20 mg per 40 ml of liquid. At this level, pollen dispersal in the medium was adequate for easy microscopic observation of the germinating grains.

Each suspension of pollen and fungi was stirred continuously on a magnetic stirrer while the Van Tieghem cells were set up to maintain a homogenous mixture. A small drop of suspension was pipetted onto a cover slip and the cover slip inverted and placed on a glass cyclinder. Each cell contained a small volume of distilled water to prevent desiccation of the culture during the incubation period. A total of thirty Van Tieghem cells were prepared for each concentration of fungus and the control. These were incubated in darkness at room temperature for 12 hours. A summary of the tests performed is presented in Table 7.

Observations of pollen germination were made at 200X with a phase contrast microscope. One observation per Van Tieghem cell was taken. A count was made of the total number of pollen grains observed and the number germinated. A pollen grain was recorded as germinated if it had developed a germination tube greater in length than its diameter. Since there were only a limited number of Van Tieghem cells available, tests were performed with each fungus individually and a separate set of controls prepared each time.

Table 7. Outline of the pollen-fungi interaction test for *Alternaria*, *Cladosporium* and *Epicoccum* using Van Tieghem cells.

Pollen Conc.	Spore Conc.	Number of Van Tieghem Cells
0.5 mg/ml	Initial	30
0.5 mg/ml	1:9 Dilution	30
0.5 mg/ml	1:99 Dilution	30
0.5 mg/ml	Control	30

The spore concentrations prepared for each test were measured with a hemacytometer and are listed in Table 8. No measurements were made of the percent germination of these spores. However, good germination (>80%) was observed in all cases.

Table 8. Spore concentrations prepared for Test 4 of the pollen-fungi interaction tests.

	Spore Concentration[1/]		
Fungus	Initial	1:9 Dilution	1:99 Dilution
Alternaria	$2.1X10^5$	$2.1X10^4$	$2.1X10^3$
Cladosporium	$1.2X10^6$	$1.2X10^5$	$1.2X10^4$
Epicoccum	$3.3X10^4$	$3.3X10^3$	$3.3X10^2$

[1/] Spores/ml

RESULTS

PART I. ISOLATION OF THE FUNGI - 1976

A large number of fungi were isolated from the white oak flowers. Of these, three genera of the Fungi Imperfecti occurred in a high frequency relative to the others. These genera were Alternaria, Cladosporium and Epicoccum. All other genera isolated were grouped together in one class (Other Fungi). The frequency of occurrence of fungi with respect to each location is shown in Table 9.

Since one third of the flowers was cultured on each isolation medium, a Chi-Square analysis was performed to determine whether any of the fungi were preferentially isolated on a particular medium. The results indicate that no medium was selective for a particular fungus (Table 10). Thus, the data from each location were combined without regard to the type of media on which the fungi were isolated.

The significance of the differences in occurrence of these fungi at each location was analyzed with a Chi-Square analysis (Table 11). Alternaria, Epicoccum and Other Fungi occurred in a significantly higher frequency at Blacksburg while Cladosporium occurred in a higher frequency at Critz.

In addition to Alternaria, Cladosporium and Epicoccum, several other genera of fungi were identified. A listing of these fungi and the number of flowers they were isolated from is presented in Table A-1 of the Appendix.

Those fungi which were sterile also occurred in low frequencies and no attempt was made to induce sporulation to facilitate identification.

Table 9. Occurrence of *Alternaria*, *Cladosporium*, *Epicoccum* and Other Fungi on the pistillate flowers of white oak (1976).

Fungus	Location			
	Blacksburg		Critz	
	Number[1/]	Percent	Number[2/]	Percent
Alternaria	317	53	57	11
Cladosporium	70	12	89	18
Epicoccum	197	33	64	13
Other Fungi	204	34	118	23

[1/] Number of flowers containing the fungus out of a total of 598[3/].

[2/] Number of flowers containing the fungus out of a total of 503[3/].

[3/] A package of 20 culture plates was misplaced while the fungi were being identified and this resulted in a loss of 39 flowers. Of these, 37 were from Critz and two from Blacksburg. Thus, there is a discrepancy in the number of flowers collected and the number reported in all results.

Table 10. Chi-square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on each isolation medium (1976).

		Culture Medium										
		PDA			CPDA			BCPDA				
Fungus	Status[1/]	Obs.	Expt.	Pct.	Obs.	Expt.	Pct.	Obs.	Expt.	Pct.	Chi Sq.	Prob.
Alternaria	P	120	125.0	33	132	124.7	36	122	124.3	33	1.01	.5-.7
	A	248	243.0	67	235	242.3	64	244	241.7	67		
		N = 368			N = 367			N = 366				
Cladosporium	P	60	53.1	16	48	53.0	13	51	52.9	14	1.68	.4-.6
	A	308	314.9	84	319	314.0	87	315	313.1	86		
		N = 368			N = 367			N = 366				
Epicoccum	P	78	87.2	21	97	87.0	26	86	86.8	23	2.79	.2-.4
	A	290	280.8	79	270	280	74	280	279.2	77		
		N = 368			N = 367			N = 366				
Other Fungi	P	117	107.6	32	109	107.3	30	96	107.0	26	2.79	.2-.4
	A	251	260.4	68	258	259.7	70	270	259.0	74		
		N = 368			N = 367			N = 366				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 11. Chi-square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi at Blacksburg and Critz (1976).

		Location							
		Blacksburg			Critz				
Fungus	Status[1/]	Obs.	Expt.	Pct.	Obs.	Expt.	Pct.	Chi Sq.	Prob.
Alternaria	P	317	203.1	53	57	170.9	11	211.97	<0.01
	A	281	394.9	47	446	332.1	99		
		N = 598			N = 503				
Cladosporium	P	70	86.4	12	89	72.6	18	7.93	<0.01
	A	528	511.6	88	414	430.4	82		
		N = 598			N = 503				
Epicoccum	P	197	141.8	33	64	119.2	13	61.76	<0.01
	A	401	456.2	67	439	483.8	87		
		N = 598			N = 503				
Other Fungi	P	204	174.9	34	118	147.1	23	14.9	<0.01
	A	394	423.1	66	385	355.9	77		
		N = 598			N = 503				

[1/] P = Fungus present on flower.
A = Fungus absent on flower.

Descriptions of the nonsporulating fungi and their frequency of occurrence are presented in Table A-2 of the Appendix.

Analysis of Critz Data

The occurrence of Alternaria and Cladosporium increased on each collection day while that of Epicoccum and Other Fungi varied with collection day at Critz. In the airspora, Alternaria and Cladosporium also increased in occurrence with each collection day while Epicoccum and Other Fungi again fluctuated. These results suggest that spores are the predominant form of the fungi on the flowers at collection time. These results are presented graphically in Figure 1.

The distribution of the fungi throughout the tree crown varied on each tree. On Tree 1, Alternaria and Epicoccum are evenly distributed throughout the crown while Cladosporium and Other Fungi occur in significantly higher frequencies in the middle and lower crown levels (Table 12). On Tree 2, Alternaria, Epicoccum and Cladosporium are evenly distributed throughout the crown while Other Fungi occur in a significantly higher frequency in the lower crown level (Table 13).

Time of collection was not a significant factor in isolating the fungi. Table 14 presents the results of a Chi-Square analysis of the difference in occurrence of the fungi at each collection time. No significant difference in fungal occurrence exists between the morning and afternoon collection periods. The difference in the occurrence of the fungi between trees was also analyzed and no significant difference was observed (Table 15).

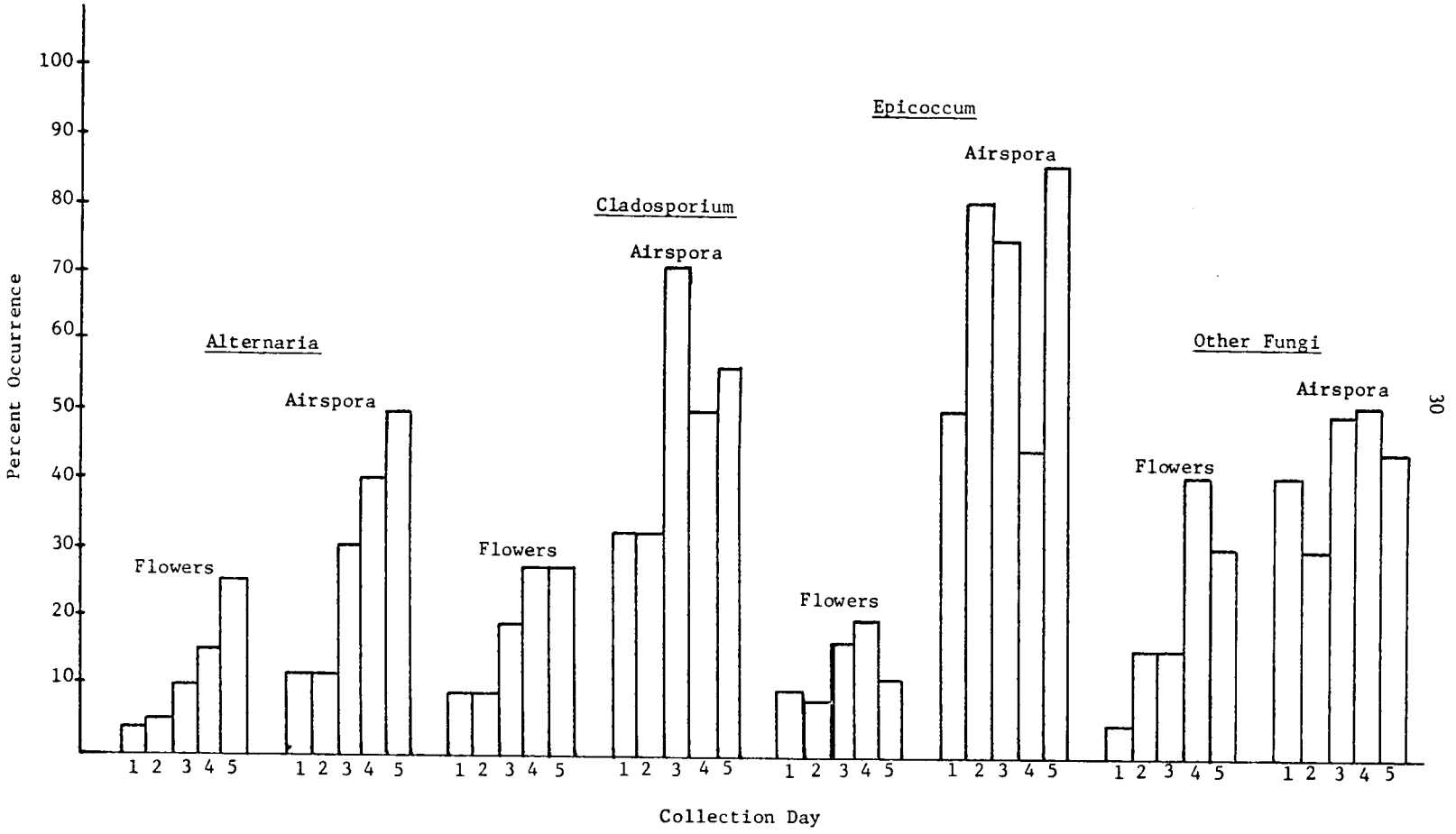

Figure 1. Comparison of the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on the oak flowers and in the airspora at Critz (1976).

Table 12. Chi-Square analysis of the difference in occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi between levels of the crown of Tree 1 at Critz (1976).

Fungus	Status[1/]	Crown Level: Upper Obs.	Upper Expt.	Upper Pct.	Middle Obs.	Middle Expt.	Middle Pct.	Lower Obs.	Lower Expt.	Lower Pct.	Chi Sq.	Prob.
Alternaria	P	11	10.6	12	11	9.0	15	10	12.4	10	1.05	.5-.7
	A	77	77.4	88	64	66.0	85	93	90.6	90		
		N = 88			N = 75			N = 103				
Cladosporium	P	7	15.9	8	20	15.3	27	21	18.6	20	10.33	<0.01
	A	81	72.1	92	55	61.5	73	82	84.4	80		
		N = 88			N = 75			N = 103				
Epicoccum	P	16	11.6	18	7	9.9	9	12	13.6	12	3.12	.2-.4
	A	72	76.4	82	68	65.1	91	91	89.4	88		
		N = 88			N = 75			N = 103				
Other Fungi	P	13	17.2	15	25	14.6	33	29	20.1	28	22.4	<0.01
	A	75	54.2	85	50	46.2	77	74	63.5	72		
		N = 88			N = 75			N = 103				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 13. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi between levels of the crown of Tree 2 at Critz (1976).

Fungus	Status[1/]	Crown Level: Upper Obs.	Upper Expt.	Upper Pct.	Middle Obs.	Middle Expt.	Middle Pct.	Lower Obs.	Lower Expt.	Lower Pct.	Chi Sq.	Prob.
Alternaria	P	12	15.5	8	11	6.3	18	2	3.2	7	5.3	0.05-.1
	A	135	131.5	92	49	53.7	82	28	26.8	93		
		N = 147			N = 60			N = 30				
Cladosporium	P	29	25.4	20	9	10.4	15	3	5.2	10	1.97	.3-.5
	A	118	121.6	80	51	49.6	85	27	24.8	90		
		N = 147			N = 60			N = 30				
Epicoccum	P	20	18	14	6	7.3	10	3	3.7	10	0.67	.6-.8
	A	127	129.0	86	54	52.7	90	27	26.3	90		
		N = 147			N = 60			N = 30				
Other Fungi	P	33	31.6	22	6	12.9	10	12	6.4	40	10.96	<0.01
	A	114	115.4	78	54	47.1	90	18	23.5	60		
		N = 147			N = 60			N = 30				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 14. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum, and Other Fungi between collection periods at Critz (1976).

Fungus	Status[1/]	Collection Period (EDT) 0830-1030 Obs.	Expt.	Pct.	1400-1600 Obs.	Expt.	Pct.	Chi Sq.	Prob.
Alternaria	P	30	29.3	12	27	27.7	11	0.04	.8-.9
	A	229	229.7	88	217	216.3	89		
		N = 259			N = 244				
Cladosporium	P	41	45.8	16	48	43.2	20	1.26	.2-.4
	A	218	213.2	84	196	200.8	80		
		N = 259			N = 244				
Epicoccum	P	34	33.0	13	30	31	12	0.07	.7-.8
	A	225	226	87	214	213	88		
		N = 259			N = 244				
Other Fungi	P	60	60.7	23	58	57.2	24	0.03	.8-.9
	A	199	198.2	77	186	186.7	76		
		N = 259			N = 244				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 15. Chi-Square analysis of the difference in occurrence of Alternaria, Cladosporium, Epicoccum, and Other Fungi on the trees at Critz (1976).

Fungus	Status[1/]	Tree 1 Obs.	Tree 1 Expt.	Tree 1 Pct.	Tree 2 Obs.	Tree 2 Expt.	Tree 2 Pct.	Chi Sq.	Prob.
Alternaria	P	32	30.1	12	25	26.9	11	0.286	.5-.7
	A	234	235.9	88	212	210.1	89		
		N = 266			N = 237				
Cladosporium	P	48	47.1	18	41	41.9	17	0.044	.8-.9
	A	218	218.9	82	196	195.1	83		
		N = 266			N = 237				
Epicoccum	P	35	33.8	13	29	30.2	12	0.103	.7-.9
	A	231	232.2	87	208	206.8	88		
		N = 266			N = 237				
Other Fungi	P	67	62.4	25	51	55.6	22	0.94	.2-.4
	A	199	203.6	75	186	181.1	78		
		N = 266			N = 237				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Analysis of Blacksburg Data

At Blacksburg the occurrence of Alternaria, Epicoccum and Other Fungi varied with each collection day while Cladosporium increased in frequency (Figure 2). Alternaria and Epicoccum occurred in much higher frequency at Blacksburg than at Critz but Cladosporium occurs less frequently (Table 16). Between tree variation in the occurrence of Alternaria and Cladosporium was not significant. However, Epicoccum occurs in a significantly higher frequency on Tree 1 while Other Fungi occur in a higher frequency on Tree 2 (Table 17).

Time of collection had no significant effect on the incidence of Cladosporium or Epicoccum at Blacksburg. Alternaria, however, occurred in a significantly higher frequency during the morning collection period while Other Fungi occurred in a higher frequency in the afternoon collection period (Table 18).

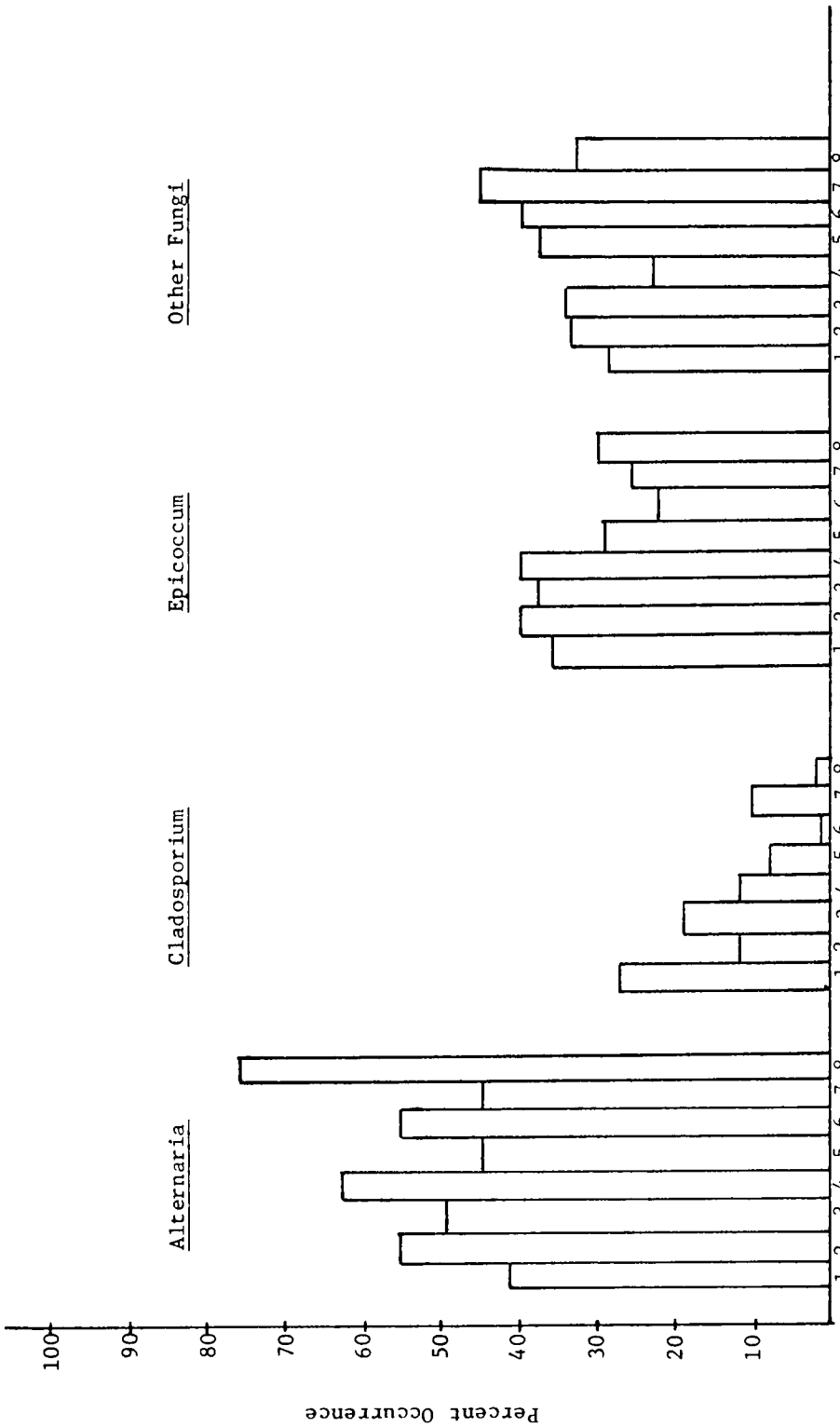

Figure 2. Frequency of occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on each collection day at Blacksburg (1976).

Table 16. Occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi in the lower crown area of the two trees sampled at Blacksburg (1976).

Fungus	Status[1/]	Tree 1 No. Observed	Tree 1 Pct.	Tree 2 No. Observed	Tree 2 Pct.
Alternaria	P	169	57	148	49
	A	129	43	152	51
		N = 298		N = 300	
Cladosporium	P	32	11	38	13
	A	266	89	262	87
		N = 298		N = 300	
Epicoccum	P	110	37	87	29
	A	188	63	213	71
		N = 298		N = 300	
Other Fungi	P	163	55	189	63
	A	135	45	111	37
		N = 298		N = 300	

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 17. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on the two trees sampled at Blacksburg (1976).

Fungus	Status[1/]	Tree 1 Obs.	Tree 1 Expt.	Tree 1 Pct.	Tree 2 Obs.	Tree 2 Expt.	Tree 2 Pct.	Chi Sq.	Prob.
Alternaria	P	169	158.0	57	148	159.0	49	3.25	.05-.1
	A	129	140.0	43	152	141.0	51		
		N = 298			N = 300				
Cladosporium	P	32	34.9	11	38	35.1	13	0.54	.4-.6
		266	263.1	89	262	264.9	87		
		N = 298			N = 300				
Epicoccum	P	110	98.2	37	87	98.8	29	4.22	<0.05
	A	188	199.8	63	213	201.2	71		
		N = 298			N = 300				
Other Fungi	P	163	175.4	55	189	176.6	63	4.25	<0.05
	A	135	122.6	45	111	123.4	37		
		N = 298			N = 300				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 18. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi between collection periods at Blacksburg (1976).

Fungus	Status[1/]	Collection Period (EDT) 0830-1030 Obs.	Expt.	Pct.	1400-1600 Obs.	Expt.	Pct.	Chi Sq.	Prob.
Alternaria	P	207	189.8	58	110	127.2	46	8.29	<0.01
	A	151	168.2	42	130	112.8	54		
		N = 358			N = 240				
Cladosporium	P	40	41.9	11	30	28.1	13	0.24	.5-.7
	A	318	316.1	89	210	211.9	87		
		N = 358			N = 240				
Epicoccum	P	116	117.9	32	81	79.1	34	0.12	.6-.8
	A	242	240.1	68	159	160.9	66		
		N = 358			N = 240				
Other Fungi	P	197	210.7	55	155	141.3	65	5.39	<0.05
	A	161	147.3	45	85	98.7	35		
		N = 358			N = 240				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

PART II. ISOLATION OF THE FUNGI - 1977

The oak flowers collected in 1977 were analyzed for the presence of Alternaria, Cladosporium and Epicoccum. All other fungi occurring on these flowers were again grouped together in one class--Other Fungi. The various genera constituting this group were not identified and thus only their combined frequency of occurrence is available. The results are presented in Table 19.

Surface sterilization of the flowers had a significant effect on the incidence of each fungus. The occurrence of the fungi on the surfaced sterilized flowers was compared to their occurrence on untreated flowers collected the same day. A Chi-Square analysis of the difference between each treatment indicates that surface sterilization causes a significant reduction in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi (Table 20). This result suggests that fungi are located primarily on the exterior of the oak flowers.

Storage of the oak flowers for four weeks prior to culturing had no significant effect on the viability of the fungi associated with them. The data from the stored flowers were compared to the results obtained from untreated flowers collected the same day. The difference in occurrence of Alternaria, Cladosporium and Epicoccum and Other Fungi in each case was not significant (Table 21).

Since there was no difference in fungal occurrence between the stored and untreated flowers, the data from each were combined. A comparison was made of the difference between the 1976 and 1977 collections at Blacksburg. Alternaria and Other Fungi decreased significantly in

Table 19. Occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on the flowers collected at Blacksburg in 1977. The results are presented according to the treatment performed on each set of flowers prior to culturing.

Fungus	Status[1/]	Treatment: No Treatment No. Observed	No Treatment Pct.	Storage No. Observed	Storage Pct.	Surface Sterilization No. Observed	Surface Sterilization Pct.
Alternaria	P	144	60	60	50	3	2.5
	A	96	40	60	50	117	97.5
		N = 240		N = 120		N = 120	
Cladosporium	P	150	63	35	29	26	22
	A	90	37	85	71	94	88
		N = 240		N = 120		N = 120	
Epicoccum	P	107	45	46	38	2	1.6
	A	133	55	74	62	118	98.4
		N = 240		N = 120		N = 120	
Other Fungi	P	182	76	80	67	7	6
	A	58	24	40	33	113	94
		N = 240		N = 120		N = 120	

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 20. Chi-Square Analysis of the difference in occurrence of Alternaria, Cladosporium, Epicoccum, and Other Fungi between surface sterilized flowers and those that were cultured immediately after collection (1977).

Fungus	Status[1/]	Non-Sterilized Flowers Obs.	Expt.	Pct.	Surface Sterilized Flowers Obs.	Expt.	Pct.	Chi Sq.	Prob.
Alternaria	P	24	13.5	20	3	13.5	2.5	18.4	<0.01
	A	96	106.5	80	117	106.5	97.5		
		N = 120			N = 120				
Cladosporium	P	77	51.5	64	26	51.5	22	44.23	<0.01
	A	43	68.5	36	94	68.5	78		
		N = 120			N = 120				
Epicoccum	P	20	11	17	2	11	1.5	16.21	<0.01
	A	100	109	83	118	109	98.5		
		N = 120			N = 120				
Other Fungi	P	35	21	29	7	21	6	22.62	<0.01
	A	85	99	71	113	99	94		
		N = 120			N = 120				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 21. Chi-Square analysis of the difference in occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on fresh compared to stored flowers at Blacksburg (1977).

Fungus	Status[1/]	Fresh Flowers Obs.	Fresh Flowers Expt.	Fresh Flowers Pct.	Stored Flowers Obs.	Stored Flowers Expt.	Stored Flowers Pct.	Chi Sq.	Prob.
Alternaria	P	60	60	50	60	60	50	0.00	>0.9
	A	60	60	50	60	60	50		
		N = 120			N = 120				
Cladosporium	P	38	36.5	32	35	36.5	29	0.177	.6-.8
	A	82	83.5	68	85	83.5	71		
		N = 120			N = 120				
Epicoccum	P	41	43.5	34	46	43.5	38	0.45	.4-.6
	A	79	76.5	66	74	76.5	62		
		N = 120			N = 120				
Other Fungi	P	67	73.5	56	80	73.5	67	2.966	.05-.1
	A	53	46.5	44	40	46.5	33		
		N = 120			N = 120				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

occurrence in 1977 while the occurrence of Cladosporium increased significantly. The level of occurrence of Epicoccum remained constant each year (Table 22).

Flowers were collected from Tree 1 and Tree 2 at Blacksburg during each year of the project and the changes in the incidence of each fungus was examined. On Tree 1, Epicoccum and Cladosporium increased significantly in occurrence in 1977 while Alternaria and Other Fungi occurred in a similar frequency each year (Table 23). On Tree 2, the occurrence of Alternaria decreased significantly in 1977 while the occurrence of Cladosporium increased in 1977. The occurrence of Epicoccum and Other Fungi remained unchanged from year to year (Table 24).

The variation between the four trees in fungal occurrence was analyzed and found to be significant. Alternaria, Cladosporium, Epicoccum and Other Fungi varied significantly in occurrence from tree to tree (Table 25).

The results of the airspora analysis made at Blacksburg in 1977 are presented in Table 26. Alternaria, Cladosporium, Epicoccum and Other Fungi all occur in high frequency.

Table 22. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum, and Other Fungi between 1976 and 1977 at Blacksburg.

Fungus	Status[1/]	1976 Obs.	1976 Expt.	1976 Pct.	1977 Obs.	1977 Expt.	1977 Pct.	Chi Sq.	Prob.
Alternaria	P	317	287.7	53	144	173.2	40	15.25	<0.01
	A	281	310.2	47	216	186.7	60		
		N = 598			N = 360				
Cladosporium	P	70	137.2	12	150	82.7	42	113.92	<0.01
	A	528	460.7	88	210	277.3	58		
		N = 598			N = 360				
Epicoccum	P	197	189.7	33	107	114.2	30	1.07	.2-.4
	A	401	408.2	67	253	245.7	70		
		N = 598			N = 360				
Other Fungi	P	352	333.3	59	182	200.7	51	6.31	<0.01
	A	246	264.7	41	178	159.3	49		
		N = 598			N = 360				

[1/] P = Fungus present on the flower.
A = Fungus absent from the flower.

Table 23. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on Tree 1 at Blacksburg between 1976 and 1977.

Fungus	Status[1/]	1976 Obs.	1976 Expt.	1976 Pct.	1977 Obs.	1977 Expt.	1977 Pct.	Chi Sq.	Prob.
Alternaria	P	169	163.6	57	44	49.4	49	1.7	.1-.3
	A	129	134.4	43	46	40.6	51		
		N = 298			N = 90				
Cladosporium	P	32	45.3	11	27	13.7	30	19.83	<0.01
	A	266	252.7	89	63	76.3	70		
		N = 298			N = 90				
Epicoccum	P	110	119.8	37	46	36.2	51	5.78	<0.05
	A	188	178.2	63	44	53.8	49		
		N = 298			N = 90				
Other Fungi	P	163	158.2	55	43	47.8	48	1.34	.2-.4
	A	135	139.8	45	47	42.2	52		
		N = 298			N = 90				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 24. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi on Tree 2 at Blacksburg between 1976 and 1977.

Fungus	Status[1/]	1976 Obs.	1976 Expt.	1976 Pct.	1977 Obs.	1977 Expt.	1977 Pct.	Chi Sq.	Prob.
Alternaria	P	148	139.2	49	33	41.8	37	4.49	<0.05
	A	152	160.8	51	57	48.2	63		
		N = 300			N = 90				
Cladosporium	P	38	59.2	13	39	17.8	43	41.8	<0.01
	A	262	240.8	87	51	72.2	57		
		N = 300			N = 90				
Epicoccum	P	87	85.4	29	24	25.6	27	0.18	.6-.8
	A	213	214.6	71	66	64.4	73		
		N = 300			N = 90				
Other Fungi	P	189	187.7	63	55	56.3	61	0.10	.7-.9
	A	111	112.3	37	35	33.7	39		
		N = 300			N = 90				

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 25. Chi-Square analysis of the difference in the occurrence of Alternaria, Cladosporium, Epicoccum and Other Fungi between the four trees sampled at Blacksburg in 1977.

Fungus	Status[1/]	Tree 1 Obs.	Tree 1 Expt.	Tree 1 Pct.	Tree 2 Obs.	Tree 2 Expt.	Tree 2 Pct.	Tree 3 Obs.	Tree 3 Expt.	Tree 3 Pct.	Tree 4 Obs.	Tree 4 Expt.	Tree 4 Pct.
Alternaria	P	44	36	49	33	36	37	23	36	26	44	36	49
	A	46	54	51	57	54	63	67	54	74	46	54	51
		N = 90			N = 90			N = 90			N = 90		
					Chi Sq. = 14.16			Prob. = <0.01					
Cladosporium	P	27	37.5	30	39	37.5	43	55	37.5	61	29	37.5	32
	A	63	52.5	70	51	52.5	57	35	52.5	39	61	52.5	68
		N = 90			N = 90			N = 90			N = 90		
					Chi Sq. = 22.44			Prob. = <0.01					
Epicoccum	P	46	26.8	51	24	26.8	27	19	26.8	21	18	26.8	20
	A	44	63.3	49	66	63.3	73	71	63.3	79	72	63.3	80
		N = 90			N = 90			N = 90			N = 90		
					Chi Sq. = 27.4			Prob. = <0.01					
Other Fungi	P	43	45.5	48	55	45.5	61	29	45.5	32	55	45.5	61
	A	47	44.5	52	35	44.5	39	61	44.5	68	35	44.5	39
		N = 90			N = 90			N = 90			N = 90		
					Chi Sq. = 20.4			Prob. = <0.01					

[1/] P = Fungus present on flower.
A = Fungus absent from flower.

Table 26. Results of the airspore analysis at Blacksburg (1977).

Date	Alternaria	Cladosporium	Epicoccum	Other Fungi
4/19/77	9[1/]	6	8	8
4/20/77	8	6	8	8
4/21/77	10	4	9	6
4/22/77	10	10	7	10
4/23/77	0	10	1	10
4/24/77	10	10	8	1
4/25/77	9	10	7	5

[1/] Number of airspora plates containing the fungus out of a total of 10.

PART III. TESTING THE INFLUENCE OF FUNGI ON POLLEN GERMINATION

The influence of fungi on pollen germination varied with each fungus tested. The results (Table 27) show both an increase and a decrease in pollen germination relative to the control for each test. Since separate controls were used for each fungus, the results from each test were analyzed separately using an Analysis of Variance procedure (ANOVA) to test for differences in the effect of each spore concentration on pollen germination. A Duncan's Multiple Range Test was also performed to determine which concentrations produced significantly different results. An alpha level of 0.05 was used in all tests.

For the genus Alternaria, changes in spore concentration did not produce significant differences in pollen germination. The data in Table 27 show similar pollen germination occurring with each treatment. An analysis of variance was performed to test the hypothesis that the differences observed are not significant. The F test was significant at a probability of 0.1 indicating that Alternaria may have a mild effect on pollen germination (Table 28).

For the genus Cladosporium, pollen germination was highest in the initial spore suspension. The analysis of variance rejects the hypothesis that the variation in pollen germination is random with a probability less than 0.01 (Table 29). Duncan's Multiple Range Test indicates that pollen germination in the initial spore suspension is significantly different from the others (Table 30).

Table 27. Results of the Van Teighem cell tests on the influence of Alternaria, Cladosporium and Epicoccum on pollen germination.

Fungus	Initial	Spore Suspension 1:9 Dil.	1:99 Dil.	Control
		Pollen Germination (%)		
Alternaria	45 ± 14.1[1/]	50 ± 9.0	42 ± 9.6	45 ± 12.1
Cladosporium	47 ± 8.8	39 ± 10.7	37 ± 12.1	40 ± 9.5
Epicoccum	55 ± 20.9	34 ± 21.0	25 ± 13.4	30 ± 15.0

[1/] ± one standard deviation.

Table 28. ANOVA for *Alternaria*-pollen interaction test.

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Treatment	3	865.19	288.39	2.18	0.0931
Error	116	15347.06	132.53		
Corrected Total	119	16239.26			

Table 29. ANOVA for Cladosporium--pollen interaction test.

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Treatment	3	1700.46	566.82	5.26	0.0021
Error	116	12491.63	107.68		
Corrected Total	119	14192.09			

Table 30. Duncan's multiple range test for the Cladosporium-pollen interaction test.

Grouping	Mean[1/]	N	Spore Concentration
A	47 ± 8.8[2/]	30	$1.2X10^6$ spores/ml
B	40 ± 9.5	30	0.0 spores/ml
B			
B	38 ± 12.1	30	$1.2X10^5$ spores/ml
B			
B	37 ± 12.1	30	$1.2X10^4$ spores/ml

[1/] Percent pollen germination.

[2/] ± one standard deviation.

For the genus Epicoccum, pollen germination was also significantly higher in the initial spore suspension. This is shown in the analysis of variance (Table 31) and Duncan's Multiple Range Test (Table 32).

Table 31. ANOVA for Epicoccum-pollen interaction test.

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Treatment	3	15675.79	5225.26	15.82	0.001
Error	116	38316.76	330.31		
Corrected Total	119	53992.56			

Table 32. Duncan's multiple range test for the *Epicoccum*-pollen interaction test.

Grouping	Mean[1/]	N	Spore Concentration
A	55 ± 20.9[2/]	30	$3.3X10^4$ spores/ml
B	34 ± 21.0	30	$3.3X10^3$ spores/ml
B			
B	30 ± 15.0	30	0.0 spores/ml
B			
B	25 ± 13.4	30	$3.3X10^2$ spores/ml

[1/] Percent pollen germination.

[2/] ± one standard deviation.

DISCUSSION

The genera Alternaria, Cladosporium and Epicoccum occur throughout the world (Ainsworth and Bisby, 1961). Several species of each genera are parasitic on crop plants, causing leaf spots, leaf molds and rots of some fruits. However, in the forest ecosystem, these fungi occur primarily as saprophytes. In regard to Quercus alba, only Cladosporium is parasitic, causing a leaf mold (USDA, 1970).

Fungi spores apparently are the primary form of inoculum on oak flowers. The related increase of Alternaria and Cladosporium on oak flowers and in the airspora at Critz (see Figure 1) and the viability of the fungi on those flowers stored for four weeks support this contention. The airspora analysis indicates that there are numerous spores of fungi in the air at the time of flowering in white oak. These spores have ample opportunity to inoculate the flowers prior to anthesis. The gummy consistency of the stigma at maturity suggests that spores would adhere to it in higher frequency than any other part of the inflorescence.

The results of the Van Tieghem cell test on the effects of fungi on pollen germination indicate that fungi are beneficial in vitro. Spores of Cladosporium and Epicoccum in high concentration stimulated pollen germination and spores of Alternaria, although they did not enhance pollen germination, did not suppress it.

The manner in which fungi enhance pollen germination is not known. Possibly, the crowding caused by the presence of the spores may provide a physical stimulus to the pollen grains. Also, the spores may be

releasing a chemical substance which induces or merely enhances pollen germination.

In the controls of the interaction tests, excellent pollen germination was observed in those instances where some pollen grains had clumped together into a small cluster. Enhancement of pollen germination under these conditions may be due either to a physical interaction between pollen grains or an increase in the concentration of a germination inducing substance released by each grain, or both. The spores of the fungi may be acting in a similar manner. However, only further experimentation will determine if this is so.

Pollen germination was substantial in each control of the interaction tests. This indicates that fungi are not essential for pollen germination. Under forest conditions, the presence or absence of fungi on the oak flowers probably has no influence on the success of fertilization. However, *in vivo* tests are needed to demonstrate if this is true or not.

CONCLUSION

A large variety of fungi are present on the pistillate flowers of white oak at the time of anthesis. Of these, three genera occur in the highest frequencies--*Alternaria*, *Cladosporium* and *Epicoccum*. Spores of these fungi occur both in the airspora and on the flowers at the time of flowering. These spores enhance pollen germination, although germination in the absence of spores is substantial.

The evidence presented here indicates that fungi do not have a major effect on the success or failure of the white oak acorn crop. However, since all tests on pollen germination were performed *in vitro*, the activity of the fungi *in vivo* is not known and warrants investigation.

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APPENDIX

Table A-1. Genera of fungi occurring in low frequency on the pistillate flowers of white oak collected in the spring of 1976.

Fungus	Occurrence[1/]	Fungus	Occurrence
Aspergillus	1	Shaeropsidales	2
Penicillium	20	Pyrenochaeta	77
Ulocladium	2	Trichocladium	2
Pestalotia	21	Phomopsis	1
Fusarium	3	Cercosporella	1
Drechslera	4	Aposphaera	1
Nigrospora	3	Sporothrix	1
Chaetomium	1	Rhizosphaera	1
Trichoderma	2	Gilmaniella	1
Curvularia	5	Pseudotorula	1
Dendryphion	1	Pithomyces	1
Actinopelte	1		

[1/] Number of flowers on which the fungus occurred out of 1101.

Table A-2. Descriptions of those fungi not producing fruiting structures.

Colony Description	Occurrence[1/]
1) light green mycelium with a scraggly, tufted appearance	3
2) light grey mycelium, very dense, resembles foam rubber	28
3) brownish-black mycelia with a felt-like texture	2
4) white mycelium, varying in texture from sparse to dense	121
5) pink mycelium that is cottony in appearance	2
6) dark green mycelium, very flat and powdery in appearance	6
7) glossy brown mycelium, resembles gelatin; wrinkled in appearance	3
8) glossy white mycelium, resembles gelatin; wrinkled in appearance	8
9) glossy red mycelium, resembles gelatin; wrinkled in appearance	1
10) glossy black mycelium, resembles gelatin; wrinkled in appearance	2
11) glossy green mycelium, resembles gelatin; wrinkled in appearance	1
12) grey mycelium, cottony in appearance	3
13) yellow mycelium, very dense, resembles foam rubber	5
14) blue-green mycelium, felt-like in appearance	3

[1/] Number of flowers on which the fungus occurred out of 1101.

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FUNGI ASSOCIATED WITH THE PISTILLATE FLOWERS OF WHITE OAK (*Quercus alba* L.) AND THEIR EFFECT ON POLLEN GERMINATION

by

Michael X. Kolpak

(ABSTRACT)

Three genera of the Fungi Imperfecti (*Alternaria*, *Cladosporium* and *Epicoccum*) were isolated in high frequency from the pistillate flowers of white oak. These genera occur worldwide and although several species of each genera are parasitic on crop plants, they are primarily saprophytic in the forest ecosystem. Of the three genera, only *Cladosporium* is parasitic on *Q. alba*, causing a leaf mold. Several other genera of fungi were isolated in low frequency from the oak flowers. Included in this group were *Penicillium*, *Pestalotia*, *Curvularia*, *Pyrenochaeta*, *Nigrospora* and *Fusarium*.

The effect of fungal spores on pollen germination was investigated. Suspensions of pollen grains and fungi spores were cultured in Van Tieghem cells. Pollen germination *in vitro* was significantly enhanced by *Cladosporium* and *Epicoccum*, but unaffected by *Alternaria*.