SPECIAL ARTICLES

PRELIMINARY NOTE ON BIRDS AS CARRIERS OF THE CHESTNUT BLIGHT FUNGUS[1]

Statements have been made by various writers that birds play a part in the dissemination of the chestnut blight fungus. Murrill[2] mentions the possible relation of birds to the disease and writes as follows: "Millions of minute summer spores emerge from day to day in elongated reddish-brown masses to be disseminated by the wind and other agencies, such as insects, birds, squirrels, etc.," also,[3] "every bird and insect that rests upon an infected spot is liable to carry the spores upon its feet or body to other trees." A few years later Mickleborough[4] mentions birds as carriers of blight spores. He says: "The minute spores are carried by wind, on the feathers of birds and the fur of squirrels." Still later Metcalf and Collins[5] say, "there is strong evidence that the spores are spread extensively by birds, especially woodpeckers." Various writers have emphasized the fact that woodpeckers frequent chestnut trees in search of insects. Fulton[6] states in a report on field work done at Orbisonia, Pa., by R. C. Walton that "woodpecker work was noted in about one tenth of the oldest lesions," but offers no conjecture as to the part played by birds, in the dissemination of the blight.

Stewart[7] says, "undoubtedly the spores are carried long distances by birds, especially woodpeckers, which visit the diseased trees, seeking borers, in the tunnels of which most of the infections occur." This statement is based on the report of Metcalf and Collins previously referred to, and is discredited by Fisher,[8] who brings out the point that this and similar statements are not based on positive evidence. There are numerous popular articles which also accuse birds of being instrumental in the spread of the blight, but these as well as the statements already quoted are based entirely on circumstantial evidence.

The first serious attempt to determine whether birds actually do carry the spores of the blight fungus were made by the field pathologists of the Pennsylvania Commission during the summer of 1912.[9] They report the testing of twenty birds as follows: eight downy woodpeckers, three creepers (kind not mentioned), two hairy woodpeckers, four flickers, and three blue jays, all with negative results. No suggestions will be made at present to account for their *negative* results, but our positive results will be presented.

During the past spring the writers have devoted considerable time to the testing of birds as carriers of the blight fungus. The first accurate analyses were made in February and the work was continued until about the middle of May. Thirty-six birds belonging to nine different species have been examined.[10] The birds were shot in the field and placed at once in sterile paper sacks for transport to the commission laboratory at the University of Pennsylvania, where the quantitative analyses were completed. Most of the birds tested were shot at either West Chester, or at Martic Forge, or in the vicinity of these places, since we wished to use the rainfall records which we were keeping at those stations. The method of making an analysis was as follows: A flask containing 100 c.c. of sterile water was emptied into a sterile moist chamber, and the bird

[1] Investigations conducted in cooperation with the Pennsylvania Chestnut Tree Blight Commission.

[2] Murrill, W. A., "A Serious Chestnut Disease," *Jour. N. Y. Botanical Garden*, 7: 146, 1906.

[3] *Ibid.*, 152.

[4] Mickleborough, J., "A Report on the Chestnut Tree Blight," Pa. Dept. of Forestry, unnumbered bulletin, p. 11, 1909.

[5] Metcalf, Haven B., and Collins, J. Franklin, "The Control of the Chestnut Bark Disease," U. S. Dept. Agr., Farmers' Bul. No. 467: 9, 1911.

[6] Fulton, H. R., "Recent Notes on the Chestnut Bark Disease," Harrisburg Conf. Rep., p. 56, 1912.

[7] Stewart, F. C., "Can the Chestnut Bark Disease be Controlled?" Harrisburg Conf. Rep., p. 43, 1912.

[8] Fisher, A. K., Harrisburg Conf. Rep., p. 103, 1912.

[9] Anderson, P. J., Elza, W. H., and Babcock, D. C., "Field Studies on the Dissemination and Growth of the Chestnut Blight Fungus," Bulletin Pennsylvania Chestnut Tree Blight Commission 3: (in press), 1913.

[10] The birds used in this work were shot by Mr. C. E. Taylor, who was formerly employed by the Pennsylvania Chestnut Tree Blight Commission.

to be tested was placed in this vessel, and its feet, tail and head and bill scrubbed vigorously with a sterile brush. The bird was then removed and the wash water shaken to secure a uniform suspension. By means of a sterile pipette, one cubic centimeter of this wash water was then added to a second flask of sterile water to make 100 c.c. Using another sterile pipette measured quantities (1 c.c. or fraction) were removed from this dilution flask and plated out in Petri dish cultures in 3 per cent. dextrose agar, plus 10. The plates were incubated as nearly as possible at 25° C. and the colonies suspected of being the blight fungus were marked at the end of four days and their later development followed. Whenever necessary they were transferred to other culture plates to verify the diagnosis. A determination was made of the number of bacterial and yeast colonies, the total number of fungous colonies, the number of colonies of the chestnut blight fungus and the number of species of other fungi represented. The original wash water was retained and centrifuged later for microscopic examination. The entire operation was carried out in a culture room with special care to exclude any sources of error. The following is a summary of results obtained up to May 12.

Name of Bird	No. Tested	No. Carrying Spores of Blight Fungus	Max. No. Spores of Blight Fungus Carried by Single Bird
Hairy woodpecker (*Dryobates villosus villosus*)..	3	0	0
Downy woodpecker (*Dryobates pubescens medianus*).....	16	13	757,074
Flicker (*Colaptes auratus luteus*).....	1	0	0
Nuthatch (*Sitta carolinensis carolinensis*).....	2	1	5,655
Golden-crowned kinglet (*Regulus satrapa satrapa*).....	1	1	6,565
Sapsucker (*Sphyrapicus varius varius*)	2	2	7,502
Brown creeper (*Certhia familiaris americana*)....	2	1	254,019
Black and white creeper (*Mniotilta varia*).....	7	0	0
Junco (*Junco hyemalis hyemalis*).....	2	1	10,000
Total.....	36	19	

The analyses show a direct relation between periods of maximum rainfall and the maximum numbers of spores obtained. During the time covered by the analyses there were four periods of heavy rainfall. The highest numbers of blight spores were invariably obtained from birds shot two to four days after a period of considerable rainfall. The maximum numbers for the four periods are as follows:

Date	No. Days after Rain	Name of Bird	No. Spores Obtained
3/19	4	Downy woodpecker...	109,022
3/29	2	Downy woodpecker...	757,074
4/18	4	Brown creeper.....	254,019
4/30	2	Downy woodpecker...	624,341

The number of species of fungi besides *Endothia parasitica* carried by the birds varied from four to fourteen as determined from the cultures. A microscopic examination of the centrifuged sediments showed, however, a much larger number, which could be detected by form, size and coloration of the spores. The total amount of wash water for each bird was centrifuged in 10 c.c. quantities and the final amount (about 2 c.c.) containing all the sediment was given a thorough microscopic examination. In sediment from birds which had yielded the high number of spores of the blight fungus it was very easy to find the *pycnospores,* but in those giving the low results the pycnospores were located with more difficulty, but they could always be found. In no cases were any ascospores found in the sediment. During the time covered by our analyses there were only five periods when ascospores were expelled in the field. The first was on March 21 and the last on April 28. The microscopic examinations substantiate the results obtained by the cultures, since the rate of development of the colonies indicated their origin from pycnospores.

To summarize, our results show that the spores of the blight fungus carried by birds are pycnospores and not ascospores and that the maximum numbers are being carried during the few days following rain periods. We are also led to the conclusion that the pycnospores carried are *brushed off* from either the normal or

diseased bark or both in the movements of the birds over these surfaces. This conclusion is supported by the fact that the birds tested were not carrying ascospores; that we have no evidence that ascospores are washed down the trees during the winter and spring months;[11] also that following a rain period pycnospores are to be found in abundance on the healthy bark below blight lesions.

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The Relation between Abnormal Permeability and Abnormal Development of Fundulus Eggs

In a previous paper[1] the suggestion was made that certain abnormalities in *Fundulus* embryos are caused by increase in permeability since osmotic pressure is not the cause and so many *different* substances have the *same* effect. It was found that the normal egg in distilled water or a "balanced" salt solution is impermeable to salts (Appendix II.). The egg appeared to be impermeable to water also, since enormous osmotic changes have no effect on it. The egg was found to contain nearly three times as much ash as sea water. The greater part of the ash is insoluble, but some of it may have been rendered so by the ashing. However, the soluble ash (3.18 per cent.) is as great as the total salts (2.84–3.29 per cent.) in the local sea water. And yet the egg develops normally, with little or no change in volume, in distilled water or in sea water that is evaporatd to one half its volume, suggesting impermeability to water. The fact that the eggs dry up when exposed to air may be taken to indicate an increase in permeability to water, due to drying of the superficial layer or plasma membrane.

In the same paper some preliminary chemical studies of the permeability were described, and the view advanced that the egg is normally impermeable to Mg ions, but since Mg was found to diffuse out of the eggs in a pure NaCl solution, this solution may have increased the permeability to Mg (p. 296). Only one experiment to test the permeability to anions was described. $MgSO_4$ solution was used, with negative results. However, the $MgSO_4$ contained too large a trace of chloride to make it possible to detect a very small diffusion of chloride from the eggs.

During the present season I was able to obtain especially pure salts, and have observed diffusion of both anions and kations from the eggs in pure solutions of these. The monstrosities produced in unbalanced salt solutions have also been studied. The experiments support the following generalizations:

1. Any solution of one or more of the salts of sea water, which is sufficiently unbalanced by other salts, *i. e.,* has a certain excess of some one kation, produces a number of types of monstrosities in *Fundulus* eggs. The types of monsters produced by the excess of one kation (*e. g.,* Na) are the same as those produced by any other (*e. g.,* K, Ca or Mg). Thus a qualitatively *specific action of a salt or ion does not exist.*

2. *These unbalanced salt solutions cause an increase in the permeability of the egg to salts.* This conclusion is based on the following data: The eggs in distilled water or in van't Hoff's solution (made with nitrates) lose no salts or ions that can be detected, except the ions of carbonic acid. On the contrary, the eggs give out salts or their ions in a mixture of NaCl and KCl or in pure solutions of the following salts: NaCl or nitrates of Na, K, Ca or Mg *in concentrations that do not kill the eggs* during the experiment. If the eggs are killed a more rapid diffusion takes place. The methods used will be published elsewhere.

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[11] Heald, F. D., and Gardner, M. W., "Preliminary Note on the Relative Prevalence of Pycnospores and Ascospores of the Chestnut Blight Fungus during the Winter," Science, N. S., 37: 916–917, 1913.

[1] McClendon, *Am. Jour. Physiol.*, 1912, XXIX., p. 290.