THE PERSISTENCE OF VIABLE PYCNOSPORES OF THE CHESTNUT BLIGHT FUNGUS ON NORMAL BARK BELOW LESIONS

R. A. Studhalter and F. D. Heald

Introduction

A study of the part taken by birds and insects in the dissemination of the chestnut blight fungus, *Endothia parasitica* (Murr.) And., has shown that both birds (6, 7) and insects (9, 10) can carry high numbers of pycnospores of this fungus. There are a number of possible sources from which these pycnospores may have been obtained, but it was thought that by far the greater number of them were brushed from both diseased and healthy chestnut bark during the normal movements of the birds and insects over these surfaces. This supposition was based primarily upon the fact, previously reported (4, 5), that pycnospores of the chestnut blight fungus, generally called the summer spores, are produced in large numbers even during the winter months, when only an occasional spore-horn can be found, and that they are washed down the trunks of trees with every rain.

It is natural to suppose that a large number of the pycnospores thus carried down the tree trunks would lodge on healthy bark below lesions. The work herein reported was undertaken in order to obtain some definite information on the numbers of pycnospores which find lodgment on normal bark, and the length of time they may remain viable after a rain.

A preliminary test of seven pieces of normal chestnut bark was made by Mr. M. W. Gardner in February, 1913, in which he obtained from 130 to 4,700 viable pycnospores per square centimeter of bark surface on the day following a rain.

Method

Pieces of smooth chestnut bark about 4 × 4 cm. in area were cut out with a flamed scalpel at varying distances below blight lesions. Without touching the bark it was dropped into an envelope which had previously been folded crosswise, placed in another envelope and

sterilized. By this means the bark was transported to the laboratory where the tests were made on the following day.

In the laboratory 100 cc. of sterile tap water was poured into a shallow sterile dish. The piece of bark to be tested was trimmed down to 2 × 3 cm., stood on end in the dish of water, held by the corners and the outer surface scraped thoroughly with a cutting needle. The scalpels and needles were flamed before using, the hands and arms washed in mercuric chloride, and the entire operation carried on in a culture room. The dish was shaken at intervals for several hours, after which time 1 cc. of its contents was transferred to a flask containing 100 cc. of sterile tap water. This flask was also permitted to stand for at least an hour, and was shaken at intervals. From this flask 1 cc. and fractions were transferred to sterile Petri dishes; and it was also found advantageous in many instances to pour several plates using fractions of 1 cc. from the wash water. Chestnut bark agar was used in all the cultures.

Incubation took place at laboratory temperatures and the plates were carefully watched for at least ten days. This was necessary on account of the fact that many of the spores of the blight fungus, although viable, would not begin to germinate at once, and colonies of *Endothia parasitica* frequently made their appearance 3 to 5 days after they were due under normal conditions.

Discussion of Results

Six series of tests, representing 36 pieces of healthy chestnut bark, were made between December 22, 1913, and June 4, 1914. Of these 36 pieces only five failed to yield positive results. The remaining 31 showed the presence of viable pycnospores of the chestnut blight fungus in numbers varying from 33 to 172,222 per square centimeter of bark surface (Table I). None of the colonies appeared in cultures at the time at which ascospore colonies usually show (3), but it should be stated that it was impossible to tell from the time of appearance of the colonies that all originated from pycnospores, on account of the frequency of delayed germination. It is a known fact, however, that the ascospores are forcibly ejected into the air, and that none are washed down the tree trunks as is the case with pycnospores (4, 5). We therefore feel certain that the colonies appearing in the cultures originated from pycnospores.

Positive results were obtained almost uniformly during December

TABLE I

Results Obtained from the Tests of Healthy Chestnut Bark Below Blight Lesions

Series 1

Collected at Martic Forge, Pa., in afternoon of 12/22/13.

Rainfall night of 12/21/13, 0.13 inch.

Date of cultures, 12/23/13.

Series 2

Collected at Martic Forge in afternoon of 1/5/14.

Rainfall 1/3–4/14 and snow (melted). 1/4–5/14, 2.11 inches.

Date of cultures, 1/6/14.

Number of Test	Distance Below Blight Lesion	No. of Viable Pycnospores of *E. parasitica* per Sq. Cm.	No. of Viable Spores of Other Fungi per Sq. Cm.	Number of Test	Distance Below Blight Lesion	No. of Viable Pycnospores of *E. parasitica* per Sq. Cm.	No. of Viable Spores of Other Fungi per Sq. Cm.
1	30 cm.	15,556	1,111	8	25 cm.	3,889	1,111
2	70 "	21,667	2,778	9	65 "	566	0
3	43 "	6,111	411	10	38 "	0	4,167
4	37 "	4,688	7,813	11	32 "	3,333	2,778
5	57 "	6,771	2,604	12	45 "	28,125	2,604
6	39 "	833	833	13	33 "	2,500	6,667
7	34 "	12,778	23,333	14	30 "	24,242	10,101

Series 3

Collected at Martic Forge in afternoon of 1/26/14.

Rainfall, 1/24/14, 1.21 inches.

Date of cultures, 1/27/14.

Series 4

Collected at West Chester, Pa., in afternoon of 1/26/14.

Rainfall, 1/24/14, 1.35 inches.

Date of cultures, 1/27/14.

15	2 cm.	4,611	1,854	22	10 cm.	633	649
16	2 "	667	1,222	23	10 "	256	51
17	1 "	708	0	24	15 "	1,975	814
18	28 "	3,764	3,667				
19	2 "	6,500	5,729				
20	1 "	1,458	5,302				
21	1 "	1,431	7,500				

Series 5

Collected at West Chester, in afternoon of 5/26/14.

Rainfall, 5/12/14, 0.56 inches.

Date of cultures, 5/27/14.

Series 6

Collected at West Chester, in afternoon of 6/5/14.

Rainfall, 6/4/14, 0.30 inches.

Date of cultures, 6/6/14.

25	0.5 cm.	0	0	34	1 cm.	17,593	0
26	1 "	33	167	35	at edge	35,185	0
27	1 "	139	641	36	2 cm.	172,222	5,556
28	0.5 "	67	33				
29	5 "	67	100				
30	1.2 "	0	0				
31	.6 "	1,181	694				
32	5 "	0	0				
33	at edge	0	417				

TABLE II

Summary of Results Obtained from the Tests of Healthy Chestnut Bark below Lesions

Series	Place of Collection, Pa.	Last Rain Before Test		No. of Days Between Rain and Time of Collection	No. of Tests Made	No. of Viable Pycnospores per Sq. Cm. of Bark		
		Date	Am't., in.			Max.	Min.	Average
1	Martic Forge	12/21/13	0.13	1	7	21,667	833	9,772
2	" "	1/3–5/14	2.11	0	7	28,125	0	8,951
3	" "	1/24/14	1.21	2	7	6,500	667	2,734
4	West Chester	1/24/14	1.35	2	3	1,975	256	955
5	" "	5/12/14	0.56	14	9	1,181	0	165
6	" "	6/4/14	0.30	1	3	172,222	17,593	75,000

and January. Snow was on the ground during one collection, and not a single spore horn was found during these two months. That many pycnospores washed down by the winter rains (4, 5) remain clinging to healthy bark below lesions is definitely shown by the first four series in Table I, and the averages in Table II.

It is to be expected, of course, that much higher results would be obtained during the summer, when pycnospores are formed in much larger numbers and spore-horns are plentiful. The single series tested in June, collected on the day following a rain of 0.30 of an inch yielded from 17,593 to 172,222 viable spores per square centimeter. The average for the June series was 75,000 per square centimeter (483,900 per square inch), while the average for the four series in December and January was 6,378 per square centimeter (41,151 per square inch).

The distances below lesions at which the pieces of bark were taken varied from 0 to 70 cm., several pieces being cut at the very edge of the lesion. It is impossible to make any definite comparison between the number of viable spores obtained at the edge of a lesion and from points further down, for no two pieces of bark were cut below the same lesion at the same time, and no two lesions can readily be compared. From the data on hand, however, it would appear that the distance below the lesion has very little influence on the number of viable pycnospores present. Since pycnospores were very plentiful at the maximum distance tested, it seems certain that positive results could have been obtained at much greater distances.

The majority of tests showed that spores of fungi other than *Endothia parasitica* were also present on the bark (Table I). It is interesting to note, however, that in the majority of the cultures a

larger number of colonies of *Endothia parasitica* appeared than of all other fungi combined, and that all of the plates from four pieces of bark developed only pure cultures of the chestnut blight fungus.

Relation to Rainfall

With the exception of Series 5, all of the collections were made not more than two days after a rain. Series 5 was collected at West Chester on May 26. The last preceding rain, 0.56 in., fell on May 12. During this interval of 14 days the weather was generally fair and warm, offering very good opportunities for the desiccation of the spores. Of the nine pieces of bark tested in this series, five yielded viable pycnospores, ranging in number from 33 to 1181 per square centimeter (Table I). Assuming that the average number of pycnospores on the day following the rain was about 75,000 per square centimeter, as was the case in Series 6 (Table II), the number which remained viable at the end of 14 days of desiccation was only a fraction of one percent of those present at the start. The significant fact, however, is that some pycnospores could withstand two weeks of desiccation in five out of nine pieces of bark tested.

After some rains the bark remains wet or moist in sheltered places for several days. Some of the bark tested was still wet at the time of collection. This was true for all of Series 2 and part of Series 1. On such areas of bark practically all of the pycnospores present would no doubt remain viable until the bark begins to dry. When the bark once begins to dry the number of viable pycnospores is probably greatly reduced by the mere act of drying, after which the further reduction will be more gradual. This statement is based upon the results obtained under certain artificial conditions (8) where the mere act of drying was found to reduce very greatly the number of viable pycnospores; once dry, the decrease was quite gradual.

It is brought out in the last mentioned work (8) that the decrease in the percentage of viable pycnospores is not as great when only a part of the mucilaginous coating is washed off, as when all the mucilage has been removed. Under natural conditions in the field it is very probable that only a small part of the mucilage is washed away by rains, for it may frequently happen that fragments of spore-horns may remain intact with nearly all of the mucilage still surrounding the spores. This condition leads us to believe that the number of pycnospores to resist desiccation is greater on the bark below lesions

than under the artificial conditions referred to, where practically no mucilage was left on the spores.

In this connection it might be stated that disease organisms have been found to resist desiccation on the seed of the host plant longer than on other material. Such is the case with the bacteria causing the black rot of cabbage (2). Spores of *Glomerella gossypii*, the cause of cotton anthracnose, retain their vitality much longer when dried on cotton seeds than on cover slips (1). Although little evidence is at hand to confirm the statement, it is not impossible that spores of *Endothia parasitica* will resist desiccation on chestnut bark much longer than on some other materials.

Practical Bearing of the Results on the Dissemination by Birds and Insects

The conclusion has been reached that birds, and especially migratory birds (6, 7), are capable of carrying large numbers of viable pycnospores of the blight fungus for considerable distances; and that insects (9, 10) may be important agents in the local dissemination of the blight. Birds were shown to be carrying pycnospores only; the same was true of nearly all of the insects from which positive results were obtained, although a very small number of ascospores were found on several beetles.

The question of the source of these pycnospores has already been touched upon in the introduction. The results herein reported bring out the following reasons in support of the belief that these pycnospores were obtained in the main, if not wholly, as a result of the brushing of birds and insects over diseased and normal chestnut bark:

1. Viable pycnospores are present in varying numbers on healthy bark below lesions after both summer and winter rains.

2. Large numbers of pycnospores can be obtained immediately after a rain, but they may be present in smaller numbers for at least two weeks.

3. The largest numbers of spores were invariably obtained from birds and insects from about two to four days after a rain, corresponding rather closely with the time at which the largest numbers of viable pycnospores are present on normal bark below lesions.

Summary

Viable pycnospores of the chestnut blight fungus were found to be present on normal bark below lesions in numbers varying from 0 to 172,222 per square centimeter of bark surface (1,111,176 per square inch).

Of the 36 pieces of bark tested, only five failed to yield positive results, and four of these five were collected 14 days after a rain.

Viable pycnospores were obtained in all but one of 24 tests made during December and January, when no spore-horns were present in the field.

An abundance of viable pycnospores was obtained at as great a distance below a lesion as 70 cm., and it appears very likely that they could be obtained at much greater distances below cankers.

Most of the tests were made one or two days after a rain; in one series, however, tested 14 days after a rain of 0.56 inch, positive results were obtained from five of the nine pieces of bark from which cultures were made.

LITERATURE CITED

1. **Edgerton, C. W.** The rots of the cotton boll. La. Agr. Exp. Sta. Bull. **137**: 1–88. *pl. 1–13*. 1912.
2. **Harding, H. A., Stewart, F. C. and Prucha, M. J.** Vitality of the cabbage black rot germ on cabbage seed. N. Y. State Exp. Sta. (Geneva) Bull. **251**: 177–194. *pl. 1*. 1904.
3. **Heald, F. D.** A method of determining in analytic work whether colonies of the chestnut blight fungus originate from pycnospores or ascospores. Mycologia **5**: 274–277. *pl. 98–101*. 1913.
4. **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. ser. **37**: 916–917. 1913.
5. **Heald, F. D. and Gardner, M. W.** The relative prevalence of pycnospores and ascospores of the chestnut blight fungus during the winter. Phytopathology **3**: 296–305. *pl. 26–28*. 1913.
6. **Heald, F. D. and Studhalter, R. A.** Preliminary note on birds as carriers of the chestnut blight fungus. Science n. ser. **38**: 278–280. 1913.
7. **Heald, F. D. and Studhalter, R. A.** Birds as carriers of the chestnut blight fungus. Journ. Agr. Research **2**: 405–422. *fig. 1–2, pl. 38–39*. 1914.
8. **Heald, F. D. and Studhalter, R. A.** Longevity of pycnospores and ascospores of Endothia parasitica under artificial conditions. Phytopathology **5**: 35–44. *pl. 2*. 1915.
9. **Studhalter, R. A.** Insects as carriers of the chestnut blight fungus. Abstract in Phytopathology **4**: 52. 1914.
10. **Studhalter, R. A. and Ruggles, A. G.** Insects as carriers of the chestnut blight fungus. Pa. Dept. Forestry Bull. [In press].