THE ASSOCIATION OF HYLURGOPINUS RUFIPES WITH THE DUTCH ELM DISEASE PATHOGEN [1]

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INTRODUCTION

There seems to be general agreement that in the United States the bark beetle *Scolytus multistriatus* (Marsham) is the principal and most effective carrier of the Dutch elm disease pathogen, *Ceratostomella ulmi* (Schwarz) Buis. The disease occurs sporadically, however, in areas of New York State where this species is not known. The identification of the disseminating and inoculating agent or agents in such areas is the objective of the studies described herein.

Most of the field observations reported in this paper were made northeast of the City of Binghamton, N. Y., in Broome and Chenango Counties, where the European elm bark beetle *Scolytus multistriatus* has not been found, but where the native elm bark beetle *Hylurgopinus rufipes* (Eich.) is prevalent. Laboratory studies correlated with the field work were made chiefly with material collected in this area.

REVIEW OF LITERATURE

Britton (4)[3] and Clinton and McCormick (7) called attention to the fact that *Hylurgopinus rufipes* might transmit the Dutch elm disease to healthy trees. They isolated *Ceratostomella ulmi* from beetles of this species taken from the bark of a diseased tree in the field, and they found that when beetles from diseased trees were confined in test tubes with twigs cut from healthy elm trees the beetles chewed the bark and the fungus fruited on the twigs. These workers observed that *Scolytus multistriatus* was not present at Old Lyme, Conn., but that *H. rufipes* was abundant on a diseased tree found there in 1934. Britton stated that *H. rufipes* may make feeding injuries on elm twigs. Kaston and Riggs (*13*) expanded these observations and suggested that the subsequent spread of the disease at Old Lyme probably was due to feeding by this species. They reported that it made feeding tunnels in the bark on branches as small as 1 inch in diameter, or

[1] Received for publication November 1, 1946.

[2] Many persons assisted with the work or made helpful suggestions. Especially substantial contributions were made by Dr. W. Howard Rankin, Anthony J. Osekosky, and B. H. Hodge of the Bureau of Plant Industry, New York State Department of Agriculture and Markets, and by Dr. Seth Pope of Cornell University. The Boyce Thompson Institute for Plant Research made available facilities for the laboratory work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 183.

Journal of Agricultural Research, Washington, D. C.

Vol. 76, Nos. 7, 8
April 1, 15, 1948
Key No. N. Y. (Cornell)–59

even smaller, but they did not observe injury to the wood. Becker (*2*, *3*) previously had reported bark tunnels on branches of that size similar to the winter tunnels in which he had occasionally observed injury to the wood.

Collins (*8*) reported an experiment in which 1 out of 11 elm trees became infected the year after adults of *Hylurgopinus rufipes* were confined on the trunks in the fall. Hagmann[4] confined fungus-bearing beetles on trees 1 to 4 inches in diameter during May and June and found infection from feeding injuries on the trunk near the origin of the first branch, or on the branches themselves, in 3 of 19 cages.[5] Collins stated that the hibernating adults, by boring into the tree in the spring, make contact with the wood, but that this is done before new wood vessels are produced and infection would not be expected at that time. He believes that adults arising later in the spring from overwintering larvae would make similar injuries to the wood after new vessels are present and that infection would then be expected. This apparent relation of time of inoculation to probability of infection seems to be supported by inoculation experiments (*6*, *15*).

Several workers have observed that the adults of *Hylurgopinus rufipes* may feed on small branches as well as large soon after they emerge, but there is a difference of opinion regarding the relative numbers of the beetles that overwinter as larvae and as hibernating adults (*12*, *14*), and therefore there is some question as to just when most of the feeding occurs.

Collins et al. (*9*) found that after emerging from diseased trees *Hylurgopinus rufipes* may carry the fungus pathogen into uninfected dead or dying elm material when making maternal galleries. Jones and Moses (*11*) isolated the fungus from substantial numbers of adults collected as they were attracted to felled healthy elm trees at different locations in New Jersey and in the Hudson River Valley in New York. The writers made studies, not reported in detail, similar to those of Collins et al. and to those of Jones and Moses, with similar results.

CULTURE STUDIES

EXPERIMENTAL PROCEDURE

Elm logs infested with the beetles were cut from diseased trees, or uninfested branches were cut from diseased trees and exposed to beetle attack in the field, and then placed in cages out of doors. The emerging adults were collected and isolation trials made for *Ceratostomella ulmi*. Cultures were made aseptically by crushing the beetles in water in petri dishes and mixing the macerated tissue with melted potato-dextrose agar.

In a similar manner, naturally infested material apparently not killed by the Dutch elm disease (such as cut wood, broken branches, and branches or trees dying because of poor environmental condi-

[4] HAGMANN, LYLE E. FEEDING HABITS AND RELATED ACTIVITIES OF THE TWO ELM SCOLYTIDS, SCOLYTUS MULTISTRIATUS (MARSHAM) AND HYLURGOPINUS RUFIPES (EICHHOFF) WITH REFERENCE TO THE SPREAD OF THE DUTCH ELM DISEASE PATHOGEN CERATOSTOMELLA ULMI (SCHWARZ) BUISMAN. 1945. [Unpublished doctor's thesis. Copy on file Cornell University library, Ithaca, N. Y.]

[5] In other work (unpublished) done at the Cornell University Dutch elm disease laboratory at Yonkers, N. Y., infection was obtained in a similar way on small potted trees.

tions) was collected in the field, placed in cages, and the emerging adults cultured.

For a more rapid method of determining the presence or absence of the fungus on beetles in the field, callow adults and pupae were collected in the field and cultured aseptically. These were obtained from cut wood and naturally occurring dead wood which apparently had not been killed by the Dutch elm disease.

A study was also made to determine whether the fungus would persist on hibernating adults. Beetles were taken from cages containing logs from which adults bearing the fungus had been cultured, placed on cut healthy logs late in the fall, and stored over the winter in small screen cages out of doors. The following spring the overwintered live beetles were dug out of the logs and cultured. Hibernating adults collected in the field during the winter and spring were also cultured.

BEETLES EMERGING FROM WOOD CUT FROM DISEASED TREES

In 1937, wood not infested with beetles was cut from diseased trees and stored at 5° C. In the spring of 1938, it was exposed in the field to beetle entry for egg laying at Armonk, Westchester County, N. Y. and on July 8 was placed in emergence cages out of doors at Yonkers.

On July 13, 1940, material infested with *Hylurgopinus rufipes* was cut from a diseased tree at Sanford, Broome County, N. Y., and on August 3 it was placed in an emergence cage out of doors. The results of cultures made from *H. rufipes* adults that emerged from the two sets of material are shown in table 1.

TABLE 1.—*Results of cultures made from Hylurgopinus rufipes adults emerging from wood from diseased trees infested in the field*

Source of wood	Period of collection of beetles for culturing	Beetles		
		Total	With *C. ulmi*	
			Number	Percent
Diseased wood infested at Armonk, N. Y.	July 26–Aug. 7, 1938	261	32	12.3
Infested wood from diseased tree at Sanford, N. Y.	Aug. 8–Aug. 16, 1940	38	30	78.9

BEETLES EMERGING FROM NATURALLY INFESTED MATERIAL FROM NONDISEASED TREES

In 1940, in connection with a survey to determine the incidence of *Ceratostomella ulmi* on *Scolytus multistariatus* emerging from naturally infested material in Westchester County during the late summer, *Hylurgopinus rufipes* also was obtained from many of the samples. Eight to 451 (usually about 150) adults of the latter species were cultured from each of 41 wood collections, which consisted of 3 logs each 4 to 5 inches in diameter and about 16 inches long. The fungus was obtained from beetles from 7 of the 41 stations.

During the same summer similar survey material was collected in the disease area in Broome and Chenango Counties. The fungus was obtained from *Hylurgopinus rufipes* adults emerging from 4 of the 11 collections.

ADULTS AND PUPAE COLLECTED IN THE FIELD

In 1941, collections from infested material were made at various stations in Broome and Chenango Counties. Adults or pupae, or both, were taken as they were available at the time the station was visited. Table 2 gives the results of cultures made from beetles collected within or at the edge of the area of disease incidence as known in 1941. Beetles carrying the fungus were obtained at 7 of the 14 stations. At these 7 stations 98 adults and pupae of a total of 1,297 gave positive cultures.

TABLE 2.—*Results of cultures made from Hylurgopinus rufipes collected from infested material in the field in 1941*

Site No.	Number of bark samples	Total area of bark sampled (square feet)	Beetles cultures (adults and pupae) Total	With *C. ulmi* Number	With *C. ulmi* Percent	Number bark samples from which beetles yielding positive cultures emerged
1	4	3.6	200	41	20.5	4
2	4	10.2	203	14	6.9	4
3	4	4.0	200	6	3.0	2
4	4	2.7	199	0	0	0
5	4	.7	198	2	1.0	1
6	1	6.0	50	0	0	0
7	1	6.0	96	14	14.6	1
8	4	18.0	201	0	0	0
9	4	15.9	200	2	1.0	1
10	4	7.9	200	19	9.5	2
11	4	4.5	212	0	0	0
12	1	5.0	99	0	0	0
13	4	7.5	201	0	0	0
14	1	10.0	24	0	0	0

PERSISTANCE OF FUNGUS ON HIBERNATING ADULTS

From September 30 to November 10, 1936, beetles emerging from material from diseased trees (cages Rh 29 and Rh 30) were introduced into cages out of doors (cages Rh 36 and Rh 37) containing wood freshly cut from healthy elm trees in which these beetles could hibernate. On April 15, 1937, beetles that had lived through the winter were collected from this wood for culturing. At that time they were just beginning to make maternal galleries. The dead beetles were not cultured. The data gathered in this experiment are presented in table 3.

TABLE 3.—*Comparison of results of cultures of adults of Hylurgopinus rufipes emerging from diseased wood in the fall and from hibernation in the spring*

Source of beetles (emergence cage No.)	Results of culturing beetles from original source material, fall of 1936: Number cultured	Number with C. ulmi	Percent with C. ulmi	Source of beetles (hibernation cage No.)	Number beetles introduced, fall 1936	Results of culturing beetles from hibernation cages, spring of 1937: Number cultured	Number with *C. ulmi*	Percent with *C. ulmi*
Rh 29	129	73	56.6	Rh 36	193	19	11	57.9
Rh 30	169	62	36.7	Rh 37	130	11	7	63.6

At intervals during the winter of 1940–41 and the spring of 1941 beetles were collected from their hibernation galleries in the field and cultured. All collections were made within the known area of disease incidence in Broome and Chenango Counties from the trunks and large branches of healthy trees. The time of year each collection was made may be roughly determined from the date on which the cultures were made. This was within 30 days of the time of collection of the beetles. Information on these experiments is given in table 4.

TABLE 4.—*Cultures of hibernating adults of Hylurgopinus rufipes collected from healthy trees in the field*

Month of culturing	Number of stations	Number of beetles	Number of cultures	Number of cultures with *C. ulcci*
November 1940	1	43	43	0
December 1940	7	188	188	4
February 1941	9	110	110	1
May 1941	8	146	36	2
June 1941	8	213	67	0

FIELD OBSERVATIONS IN THE UPSTATE AREA OF DISEASE INCIDENCE

Following the discovery in 1939 of the Dutch elm disease in Broome County, N. Y., by scouts of the United States Department of Agriculture, detailed observations were made on many of the infected trees found in that area. These studies were similar to those made in the Hudson River Valley (10). Since *Scolytus multistriatus* is not known to occur in the Broome County area, and it has been shown that *Hylurgopinus rufipes* in that area carries the fungus and can inoculate healthy trees, it is probable that the latter species inoculated all the diseased trees that were observed.

In 1940, 56 diseased trees were observed at 30 sites. In 34 of these trees it was estimated that at least 10 percent of the branches were dead and had harbored or were still harboring *Hylurgopinus rufipes*. In addition, unsuccessful maternal galleries had been attempted in at least 4 other trees.

In 1941, 30 diseased trees were observed at 22 sites. In 15 of the trees, at least 10 percent of the wood was dead.

At each site an attempt was made to determine the year when each tree first became infected and the nearest source of beetles. This particular observation was independent of the subsequent history of the diseased trees. That is, the beetle emergence referred to here occurred prior to infection of the trees.

Table 5 lists the types of wood which contained the observed beetle sources and the distances from the diseased trees to the nearest probable beetle sources. In some instances no source was found. The observed emergence usually, but not necessarily always, occurred at a time when the beetles could have made the specific inoculation involved. In most of the diseased trees cited as being their own sources of beetles, the branches from which beetles had emerged were killed by unfavorable soil conditions or other environmental factors. Such trees are included among those listed as being within 100 feet of the source of beetles.

TABLE 5.—*Types of beetle sources and number of diseased trees associated with them, and distances from beetle sources to diseased trees*

Type of beetle source and distance from source to diseased trees	Number of diseased trees associated with beetle sources		
	1940	1941	Total
Types of beetle source:			
Branch of same diseased tree [1]	24	5	29
Other diseased trees [2]	8	2	10
Dead trees or dead branches in other trees	21	10	31
Broken-off branches or logs	0	5	5
Source unknown	3	8	11
Distances from beetle source to diseased trees:			
100 feet or less	36	15	51
Between 100 and 250 feet	12	7	19
Between 250 and 500 feet	5	0	5
Unknown	3	8	11
Total trees observed	56	30	86

[1] Beetles emerged before tree became infected.
[2] Only sources of beetles observed that are believed to have been diseased branches. All others listed in this table were in material not killed by the Dutch elm disease.

Feeding tunnels as described by Kaston (*12*) and others were observed on many of these trees. Many tunnels reached and injured the wood, especially on the smaller branches, ¾ inch to 4 inches in diameter, where the bark was thin. Injury to the wood was also observed apparently arising from hibernation galleries as described by Becker (*2*). The appearance of the injury and adjacent recent extension to the hibernation tunnel when seen in the spring usually indicated that wood injury was made at that time.

A rather high proportion of the diseased trees contained live colonies of *Hylurgopinus rufipes*. It is suggested that many such trees were inoculated by the beetles in connection with oviposition in portions which were still in a state of suitable physiological activity to permit infection at the time of the actual or attempted oviposition. There are no published data demonstrating that infection results from tunnels made for egg laying, but it has been shown that trial entries by *Scolytus multistriatus* may result in effective inoculations.[6] On trees with completed galleries of *H. rufipes*, trial entries or feeding tunnels which injured the wood were sometimes found in adjacent, somewhat more vigorous branches.

The incidence of diseased trees in this area was very low, an average of one in 5 square miles in 1940 and one in 10 square miles in 1941. Frequently, the disease attacked the weakest tree in a group. That is, trees receptive to beetle entrance became diseased first. Later, perhaps the following year as a rule, more vigorous trees in the neighborhood became diseased. Probably, in diseased tree associations of this kind the first inoculations were made by trial entries for egg laying,

[6] Unpublished work at Cornell University Dutch elm disease laboratory at Yonkers, N. Y.

as in observations reported by Collins (*8*). Subsequently, the bettle population was built up to such an extent that the relatively small percentage making injuries to the wood in feeding activities was sufficient to make a few effective inoculations.

DISCUSSION

The following discussion is an attempt to interpret the results of these studies, and the low incidence of the Dutch elm disease in the area northeast of Windsor, N. Y., where the bark beetle species *Scolytus multistriatus* is absent but *Hylurgopinus rufipes* is present, in the light of published information on the life history and habits of the latter species. Since *Ceratostomella ulmi* was obtained from *H. rufipes* adults taken from cut or broken elm branches originating in nondiseased trees, the breeding activities of this species is probably a means of introducing the fungus into new areas. The abundance of the fungus in such material in the field was substantially as great as that found by Collins et al. (*10*) associated with *S. multistriatus;* that is, it was obtained from beetles from similar percentages of wood samples, and the number of fungus colonies per beetle was about the same.

Probably the time when inoculations are most likely to cause destructive infections is early in the spring, but not until after new xylem vessels are produced. Any one of three methods of inoculation by *Hylurgopinus rufipes* might be operative at this time. Many of the wood injuries resulting from extensions of hibernating tunnels and from attempted entries by overwintered adults may be made before new xylem vessels are produced and such injuries would not be likely to result in infection. However, according to Kaston (*12*), some of these beetles do not leave their hibernation tunnels until late May or June, and infection should result from inoculations made by them. Feeding by beetles that arise from overwintering larvae has been shown to cause infections. There is also the possibility that effective inoculations may be made in the middle of the summer or later by this species. The work of Banfield (*1*) indicates that the movement of the fungus spores when introduced into trunks or large branches during the latter part of the growing season is slower and less extensive than earlier in the season. But the movement is extensive enough to make it appear plausible that inoculations made either by trial entry or by feeding by *Hylurgopinus rufipes* late in the season might be effective. Furthermore, since this species feeds in larger branches, inoculations made in the summer would be more likely to result in infection than would those made at the same time by the feeding of *Scolytus multistriatus* in the small twigs. One of the writers (Tyler)[7] found that hypodermic inoculations on the trunks late in the season were more successful than those made on the twigs at the same time.

Regardless of the several possible methods by which *Hylurgopinus rufipes* may make inoculations, the number of known infections in areas in New York State where it is abundant and *Scolytus multistriatus* is absent are relatively few (*5*). This fact is undoubtedly associated with two well-defined relationships, namely, that (1) egg-laying trial

[7] Unpublished data.

tunnels apparently are usually made in branches with insufficient vigor for the fungus to invade the live parts of the trees, and (2) wood injuries made in connection with hibernation and feeding activities are apparently not sufficiently frequent, or are made at the wrong time of the year, to account for many inoculations. Detailed observations by the writers on nearly 100 diseased trees and more casual observations over a period of several years on many more have failed to reveal abundant injuries of any of these types on sound branches. When compared with the deep-feeding injuries to the twigs made by *S. multistriatus* they would be considered as rare.

SUMMARY

The bark beetle *Hylurgopinus rufipes* may carry the Dutch elm disease pathogen when emerging from infested wood originating in diseased trees, cut elm wood, or dead branches and trees. The fungus may survive the winter on hibernating adults. It seems to be adapted to a congenial association with this native beetle in dead elm wood.

Field observations on the incidence of diseased trees in New York State indicate that *Hylurgopinus rufipes* has been very much less effective as an inoculating agent in the area studied than *Scolytus multistriatus* in the Hudson River Valley. One reason for this is the fact that *H. rufipes* is much less likely than *S. multistriatus* to make injuries that reach to and into the wood of sound trees and branches.

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U. S. GOVERNMENT PRINTING OFFICE: 1948

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