ISOLATION OF CERATOSTOMELLA ULMI FROM INSECTS ATTRACTED TO FELLED ELM TREES [1]

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INTRODUCTION

The role of insects in the dissemination of *Ceratostomella ulmi* Buisman, the fungus that causes the Dutch elm disease, seems to be twofold. The insect contaminated with the fungus may feed directly on a living elm tree and thereby cause infection, or it may establish *C. ulmi* in dead or dying elm wood, whence its progeny or other insects may carry the fungus when they emerge. A knowledge of what insects are involved, and of their relative importance, is an essential part of the foundation upon which measures for the eradication and control of the disease must be based.

During the years 1936–39 insects were collected from recently felled elm trees at selected locations in New Jersey and New York. For the most part these insects were species known to breed in elms. They were later cultured to determine what species were carrying *Ceratostomella ulmi,* and to find the percentage of individuals of each species that were contaminated with the fungus. The results of this investigation are presented in this paper.

METHODS OF COLLECTING AND CULTURING INSECTS

The insects were taken from the surface of the bark of healthy American elms (*Ulmus americana* L.) that had been felled and placed horizontally on supports about 2 feet from the ground. They were collected individually in new gelatin capsules, care being taken to prevent contamination. The insects were sent by mail daily to the Morristown, N. J., laboratory, where they were identified without being removed from the capsules and refrigerated at 5° C. or lower temperatures until they were cultured.

The insects were cultured individually according to the following method, a modification of that described by Walter.[4] A Petri dish containing two pieces of filter paper and a chip from a small twig of seasoned elm was autoclaved for 20 minutes at 15 pounds' pressure and moistened with 4 cc. of sterile distilled water. The insect was then crushed on the chip with a sterile forceps, and the Petri dish was

[1] Received for publication February 25, 1942. The investigation reported was a cooperative one between the Dutch Elm Disease Eradication unit and the Division of Forest Insect Investigations of the Bureau of Entomology and Plant Quarantine, and the Division of Forest Pathology of the Bureau of Plant Industry. Members of the Dutch Elm Disease Eradication unit cut, placed, and later removed the elm trees from which the insects were collected, and employed men to collect and culture these insects.

[2] Died Feb. 22, 1941.

[3] The writers wish to acknowledge their indebtedness to those members of the three cooperating agencies who assisted in this work in various ways, especially the following members of the Bureau of Entomology and Plant Quarantine: To C. H. Hoffmann for determining many of the insects, to D. O. Wolfenbarger for assistance in supervising the field work in 1936, to R. R. Whitten for advice regarding the sampling method used in 1939, and to A. E. Lantz for preparing the figures that accompany this article.

[4] WALTER, J. M. TECHNIQUE ADVANTAGEOUS FOR THE ISOLATION OF CERATOSTOMELLA ULMI FROM BARK BEETLES. (Abstract) Phytopathology 25: 37–38. 1935.

incubated for about 20 days at 10° to 15° C. Since the typical fruiting structure of the imperfect stage of *Ceratostomella ulmi* is a coremium about 1,200 to 1,500μ in height, it is fairly conspicuous, especially when observed under a binocular dissecting microscope. Transfers were made from the coremial heads to malt or potato-sucrose agar plates with a sterile needle. Identification of *C. ulmi* was made by microscopic examination of the colonies produced by these transfers. This procedure was necessary because coremia-forming fungi other than *C. ulmi* were sometimes present in the cultures.

INVESTIGATIONS IN 1936

In 1936 three felled trees were installed on May 18 in sunny situa-

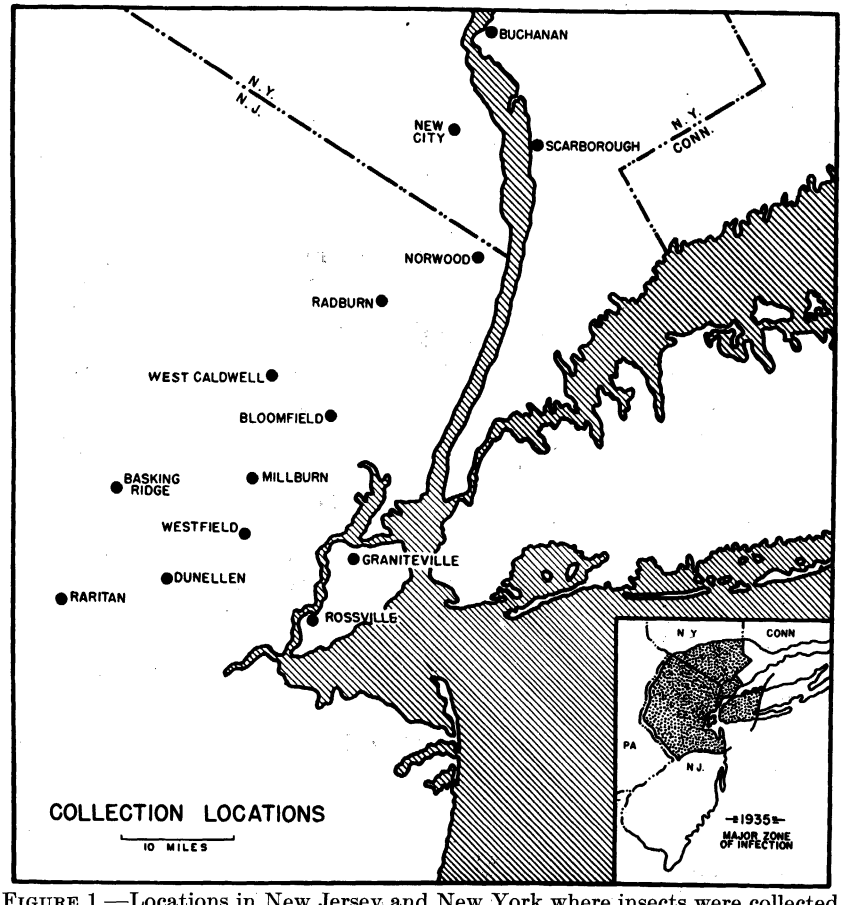

FIGURE 1.—Locations in New Jersey and New York where insects were collected. Insert shows major zone of Dutch elm disease infection as known in December 1935 and within which the insect collections were made.

tions at each of six places in New Jersey where elm trees affected by the Dutch elm disease had been removed previously in some numbers. These locations were in Basking Ridge, Westfield, Bloomfield, Radburn, Millburn, and West Caldwell (fig. 1). After 2 weeks one of the trees at each location was removed, burned, and replaced by a newly felled tree. This procedure was repeated every 2 weeks so that three

felled trees were exposed at all times at each location and no tree was left longer than 6 weeks.

A man was assigned to each location with instructions to collect all adults seen on the bark of the trees. It might be expected that numerous individuals of species having no intimate connection with elm would be taken, but as a matter of fact most of the insects taken were of species known or suspected to develop in elm, either by feeding in the wood and bark or as parasites and predators on insects breeding in elm.

The collectors worked at all locations from May 25 to September 9, and some of them for a few days before or after this period. Each man was on duty on week days from 8 a. m. to 4:30 p. m. (eastern daylight-saving time) and on Saturdays from 8 a. m. to noon, except that once each week his hours were from 4 p. m. to midnight. At night the men used electric lanterns to aid them in finding insects on the trap trees, but no trapping devices other than the felled trees were employed.

TABLE 1.—*Isolation of Ceratostomella ulmi from insects collected at six selected locations in New Jersey in 1936*

Insect	Insects cultured	Insects from which *C. ulmi* was isolated
	Number	*Percent*
Coleoptera:		
Scolytidae:		
Scolytus multistriatus (Marsh.)	7,209	6.9
Scolytus sulcatus Lec.	1	0
Hylurgopinus rufipes (Eich.)	139	4.3
Xylosandrus germanus Bldfd.	826	.2
Xyleborus sp.	25	0
Chramesus hicoriae Lec.	1	0
Other Scolytidae	1	0
Bostrichidae: *Xylobiops basilaris* (Say)	114	1.8
Cerambycidae:		
Saperda tridentata Oliv.	78	0
Neoclytus acuminatus (F.)	555	0
Xylotrechus colonus (F.)	89	0
Other Cerambycidae	2	0
Buprestidae:		
Chrysobothris femorata (Oliv.)	202	0
Anthaxia viridifrons Gory	83	0
Other Buprestidae	2	0
Curculionidae:		
Magdalis armicollis (Say)	1,419	.1
Magdalis barbita (Say)	338	0
Magdalis inconspicua Horn	474	0
Conotrachelus anaglypticus (Say)	143	.7
Other Curculionidae	2	0
Cleridae:		
Enoclerus quadriguttatus (Oliv.)	9	0
Chariessa pilosa (Forst.)	15	0
Other Cleridae	1	0
Histeridae:		
Platysoma coarctatum Lec.	3	0
Other Histeridae	2	0
Lampyridae	27	0
Nitidulidae: *Colopterus semitectus* (Say)	1	0
Corylophidae: *Molamba* sp.	1	0
Melandryidae: *Synchroa punctata* Newm.	9	0
Elateridae	37	0
Tenebrionidae	1	0
Chrysomelidae: *Microrhopala vittata* (F.)	4	0
Hymenoptera:		
Xiphydriidae: *Xiphydria* sp.	2	0
Braconidae: *Capitonius saperdae* (Ashm.)	5	0
Formicidae	58	0
Hemiptera:		
Membracidae:		
Stictocephala lutea (Walk.)	65	0
Other Membracidae	3	0
Pentatomidae: *Brochymena* sp.	1	0
Total	11,947	

The insects collected and cultured in 1936, as well as the percentages yielding *Ceratostomella ulmi*, are given in table 1. The species showing the highest percentage of infection were *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eich.), and the fungus was isolated from four other species. The numbers of *S. multistriatus* and *H. rufipes* collected at each of the six locations, and the percentages from which *C. ulmi* was obtained, are shown in table 2.

Figure 2 shows graphically how the numbers of *Scolytus multistriatus* taken during weekly collection periods at all locations, and the percentages of these beetles found to be contaminated with *Ceratostomella ulmi*, varied throughout the season. Only five beetles were collected during the May 24–31 and the September 13–19 periods in 1936, and it is on these limited numbers that the indicated 20 percent of contaminated beetles is based. The small number of *S. multistriatus* adults taken between July 12 and 25 is interpreted as being due to the fact that this period fell between the peaks of abundance of adults of the first and second generations.

The number of collected adults of the various species known to breed in elm cannot be used as a basis for estimating the comparative abundance of these species, even in the immediate vicinity of the trap trees. It is certain, for instance, that had the trees been installed earlier in 1936 and in the subsequent years in which the investigation was conducted, more *Hylurgopinus rufipes* would have been collected. Sixty percent of the insects collected were *Scolytus multistriatus*. This species is recognized as the most important insect vector of the Dutch elm disease fungus in this country.

Since the sex of *Scolytus multistriatus* adults can be determined readily, it was deemed advisable to ascertain whether a greater percentage of one sex than of the other was carrying the fungus. The sex was determined before the beetles were cultured. Males composed 71.2 percent of the total number, no doubt because the males run about more on the surface of the bark than do the females and were thus more often seen by the collectors. *Ceratostomella ulmi* was isolated from 6.9 percent of the males and from 7.1 percent of the females.

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TABLE 2.—*Isolation of Ceratostomella ulmi from Scolytus multistriatus and Hylurgopinus rufipes collected at selected locations in 1936, 1937, 1938, and 1939*

Location	From *Scolytus multistriatus*								From *Hylurgopinus rufipes*							
	Insects collected in—				Insects giving *C. ulmi* in—				Insects collected in—				Insects giving *C. ulmi* in—			
	1936	1937	1938	1939	1936	1937	1938	1939	1936	1937	1938	1939	1936	1937	1938	1939
New Jersey:	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Basking Ridge	620	2,685	1,960	1,400	8.5	6.8	11.3	6.75±1.55	72	566	84	356	4.2	2.6	1.2	0.8
Westfield	1,551	1,625	8,940	9,357	6.9	5.4	12.7	1.75±0.66	0	1	144	107	0	0	4.2	0
Bloomfield	2,461	3,925	2,162	5,143	4.9	5.7	3.9	6.00±1.28	1	5	6	7	100.0	0	0	14.3
Radburn	497	542	5,281	1,020	3.0	.9	2.3	3.00±1.33	0	0	0	1	0	0	0	0
Millburn	1,414	972	6,339	3,517	9.5	5.7	8.7	9.25±1.41	11	6	15	51	0	0	0	0
West Caldwell	666	47	8,510	6,167	10.1	19.2	5.1	7.50±0.92	55	34	25	32	3.6	0	8.0	0
Total or average	7,209	9,796	33,192	26,604	6.9	5.8	7.7	5.71±2.51	139	612	274	554	4.3	2.4	3.3	.7
Norwood		410				2.9				0				0		
Raritan		2,321				5.1				43				2.3		
Dunellen		380				3.9				0				0		
New York:																
New City		280				3.2				63				1.6		
Scarborough		1,218				2.3				122				.8		
Buchanan		827				.8				65				0		
Rossville		351				.8				0				0		
Graniteville		2				0				0				0		
Grand total or average		15,585				4.8				905				2.0		

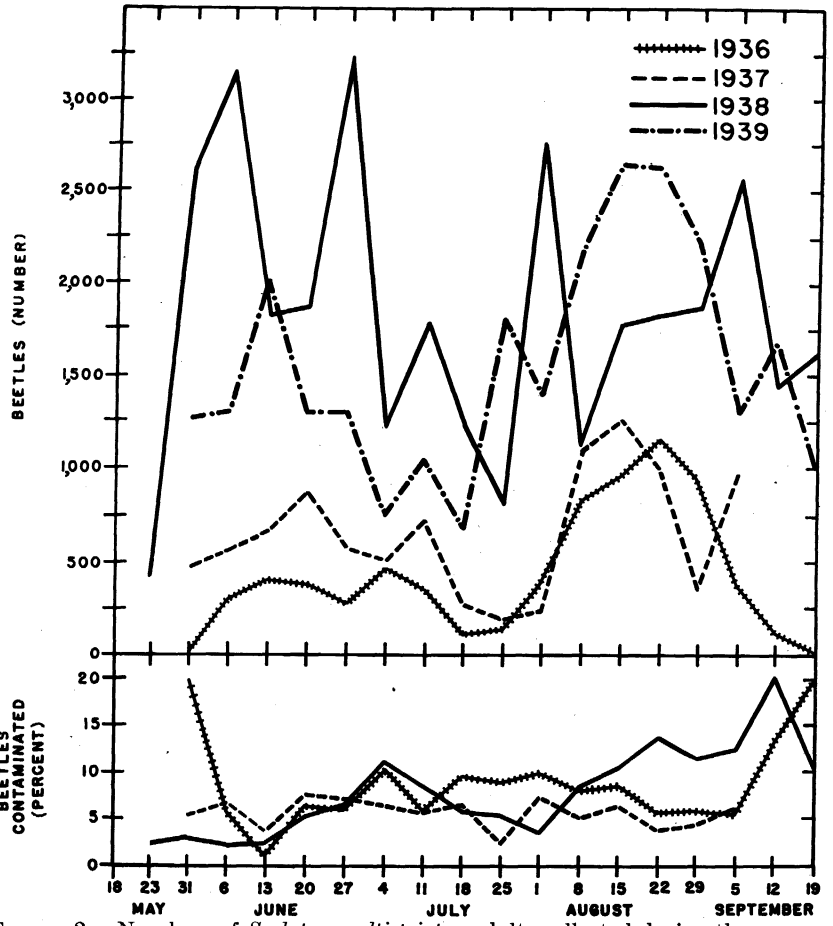

FIGURE 2.—Numbers of *Scolytus multistriatus* adults collected during the seasons of 1936–39, and the percentages of these beetles contaminated with *Ceratostomella ulmi* during the first 3 years. Collections from six locations in New Jersey have been combined for the periods indicated.

INVESTIGATIONS IN 1937

Freshly cut elm trees were installed on May 17, 1937, at the same places in New Jersey in which insect collections had been made during 1936. Trees were also installed at eight additional places—Norwood, Raritan, and Dunellen, N. J., New City, Scarborough, and Buchanan, N. Y., and Rossville and Graniteville on Staten Island, N. Y. (fig. 1). Four trees were placed at each location, three in a sunny situation and the fourth where it would be shaded most of the time. The trees in the sunny situation were later removed and replacements made as in 1936. The tree in the shady location was removed every 4 weeks and another put in its place.

Since in 1936 *Ceratostomella ulmi* was isolated more frequently from *Scolytus multistriatus* and *Hylurgopinus rufipes* than from the other species, and since these species are considered to be the most important

insect carriers of *C. ulmi* in the United States,[5] the collections of 1937 were limited to these two species.

The collectors began work on May 17, but they captured no bark beetles until May 24. At the six sites used in 1936 collecting was continued until September 5, but at the eight additional sites it was discontinued on July 14. The men were at each location on alternate days only, instead of daily as in 1936.

No night collecting was done in 1937. The men worked from 9 a. m. until 6 p. m., for it had been found in 1936 that 8 a. m. was early for insect activity and that activity continued after 4:30 p. m. Therefore, they probably captured more insects during any one day than had the hours been the same as in 1936. Because of this change in hours, because collections were made only on alternate days at any one place in 1937, and for other reasons the difference in numbers of *Scolytus multistriatus* or *Hylurgopinus rufipes* taken during the 2 years does not represent yearly fluctuations in abundance of either species.

The methods of identifying and culturing the insects in 1937 were similar to those in the previous year. The numbers of *Scolytus multistriatus* and *Hylurgopinus rufipes* taken at each location and the percentages of each yielding *Ceratostomella ulmi* are shown in table 2. As in 1936, *C. ulmi* was isolated from a higher percentage of *S. multistriatus* than of *H. rufipes*. The combined number of *S. multistriatus* taken at the six locations during approximately weekly periods and the percentages of these beetles from which *C. ulmi* was isolated are indicated in figure 2.

The sex ratio of *Scolytus multistriatus* approached that in 1936. In 1937, 76.6 percent of this species were males. *Ceratostomella ulmi* was isolated from 6.0 percent of the males and from 5.0 percent of the females.

INVESTIGATIONS IN 1938

In 1938 four trap trees were again put in place at each of the six sites used in 1936 and 1937. Three trees were again placed in a sunny situation and one in a shaded position. The schedule for removing the trees and replacing them by others was the same as in 1937. Collectors were at each location on alternate days from May 16 to September 17. Their hours were the same as in 1937, and they again collected only *Scolytus multistriatus* and *Hylurgopinus rufipes*.

Table 2 includes the numbers of the two species taken at each of the six locations, and the percentages of each from which *Ceratostomella ulmi* was obtained. Figure 2 indicates the combined number of *Scolytus multistriatus* taken at the six sites during intervals of approximately a week throughout the collecting season, and the percentages of these beetles from which *C. ulmi* was cultured. No separation of the sexes of *S. multistriatus* was made in 1938.

From figure 2 it will be noted that many more *Scolytus multistriatus* were collected in 1938 than in either of the 2 previous years, particularly in the early part of the season. The marked increase is believed to be due to the fact that numerous elms near four of the sites had been treated with dry copper sulfate late in 1936 or early in 1937. These elms were of small value, and the Dutch Elm Disease Eradica-

[5] MAY, C., and COLLINS, C. W. THE DUTCH ELM DISEASE IN THE UNITED STATES AND ITS INSECT VECTORS. West. Shade Tree Conf. Proc. 5: 49–54. 1938.

tion unit treated them with the chemical for the purpose of killing them and thus reducing the number to be inspected for external symptoms of the disease.[6] When the work was done it was not expected that bark beetles would attack these chemically treated trees. They did, however, and many of the *S. multistriatus* adults that were taken from the trap trees in 1938 may have originated in these trees. Conversely, it is also probable that the treated trees influenced the number of *S. multistriatus* collected from the trap trees in 1937, because the trees treated with copper sulfate were more attractive to the beetles than were the trap trees.

Figure 2 also indicates that a larger percentage of *Scolytus multistriatus* beetles collected during the latter part of 1938 were contaminated with *Ceratostomella ulmi* than of the beetles collected earlier. Most of these contaminated beetles were taken from one location, where the collections were comparatively large.

INVESTIGATIONS IN 1939

The procedure followed in installing the trap trees and collecting insects in 1939 was the same as that used in 1938. The collectors reported for duty on May 22 and worked until September 16.

It was impossible to culture all the *Scolytus multistriatus* beetles collected in 1939. Consequently, the capsules containing those beetles taken at each location were thoroughly mixed, and 20 samples of 20 beetles each were drawn at random and then cultured in the usual manner.[7] The culture results were analyzed statistically according to standard methods. Table 2 indicates the number collected at each location and the percentages from which the fungus was obtained.

Figure 2 shows the total number of *Scolytus multistriatus* taken at the six sites at intervals of approximately a week. Since the collections for the entire season were combined, it is impossible to show the weekly variation in the percentages of beetles carrying the fungus, as has been done for previous years.

All *Hylurgopinus rufipes* adults collected in 1939 were cultured. The number taken at each location and the percentages of each from which the fungus was obtained are given in table 2.

SUMMARY

Experiments were conducted from 1936 to 1939 to ascertain what species of insects were carrying the Dutch elm disease fungus (*Ceratostomella ulmi* Buisman) and from what percentage of each of these species the organism could be isolated. Insects attracted to felled healthy elm trees placed at several locations in New Jersey and New York were collected, identified, and cultured.

In 1936 all adult insects found on the surface of the bark of felled elms at six sites in New Jersey were collected and cultured. They included identified individuals of 23 species and many other specimens that were determined only to family or genus. *Ceratostomella ulmi* was isolated most frequently from two species of elm bark beetles, *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eich.). It was also isolated from four other species of Coleoptera, namely,

[6] BREWER, E. G., and MIDDLETON, W. DUTCH ELM DISEASE ERADICATION; JAPANESE BEETLE CONTROL; EUROPEAN CORN BORER AND GYPSY MOTH CERTIFICATION. Jour. Econ. Ent. 31: 577–583. 1938.

[7] In 1939, all the culture work was done under the supervision of L. M. Fenner and E. G. Kelsheimer, of the Bureau of Entomology and Plant Quarantine.

Xylobiops basilaris (Say), *Conotrachelus anaglypticus* (Say), *Xylosandrus germanus* Bldfd., and *Magdalis armicollis* (Say).

Because of the results of the 1936 experiment, and because *Scolytus multistriatus* and *Hylurgopinus rufipes* are considered the most important insect vectors of *Ceratostomella ulmi* in the United States, only adults of these two species were cultured in 1937, 1938, and 1939. They were collected at the same six locations as in 1936 Many more *S. multistriatus* than *H. rufipes* were taken. *C. ulmi* was obtained from 6.9, 5.8, 7.7, and 5.71 percent of the *S. multistriatus*, and from 4.3, 2.4, 3.3, and 0.7 percent of the *H. rufipes*, cultured in 1936, 1937, 1938, and 1939, respectively. The percentage of beetles contaminated with *Ceratostomella ulmi* varied considerably at different locations in the same year and at the same location in different years. In 1937 *Scolytus multistriatus* and *H. rufipes* collected at eight additional locations in New Jersey and New York were cultured.

In 1936 and 1937 the sex of the *Scolytus multistriatus* adults was determined before they were cultured. Males composed 71.2 percent of the adults in 1936 and 76.6 percent in 1937. In 1936 *Ceratostomella ulmi* was cultured from 6.9 percent of the males and from 7.1 percent of the females; in 1937 the percentages were 6.0 and 5.0, respectively.