Spread and Increase of *Ceratocystis ulmi* with Cultural Characteristics of the Aggressive Strain in Northeastern North America

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ABSTRACT

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Elm trees with Dutch elm disease in the state of Vermont and in the town of Millinocket, ME, were systematically sampled for infection by *Ceratocystis ulmi* in 1980 and 1983. Isolates of *C. ulmi* were classified as either the aggressive or nonaggressive strain on the basis of growth rate and cultural morphology. Results indicated that these two subgroups are isolated in nature. Compared with an earlier survey in 1977, the relative number of nonaggressive isolates declined with each successive survey in both areas, suggesting that this strain will soon disappear from these areas.

It is now established that *Ceratocystis ulmi* (Buism.) C. Moreau, the cause of Dutch elm disease, exists as three reproductively isolated subspecific groups (5). These include two races, the Eurasian (EAN) and North American (NAN) of the aggressive strain (AG) and a nonaggressive strain (NA) (2,9).

Studies in Britain showed that AG (NAN race) and NA could be distinguished on the basis of their rate and extent of colonization of such moderately resistant species as *Ulmus procera* Salisb. and also by their different growth rates and cultural morphology on 2% Oxoid malt extract agar (9). Subsequent trials in the United States, Holland, and Britain further established the correlation between growth rate-colony morphology and pathogenicity (10). Although the genes that control pathogenicity have yet to be shown to be the same as those that regulate growth and morphology, there is evidence that ". . . the characteristic culture morphology of the aggressive strain is caused by the pleiotropic effect of loci responsible for the high level of pathogenicity" (1). Because of their cultural and physiological differences, the two strains probably should be considered subspecies (4).

A study in 1977 revealed that isolates of *C. ulmi* from diseased elms in central and eastern North America could be separated readily into two subgroups whose growth rates and cultural morphology were characteristic of AG and NA (11). The predominance of AG in the central states, and the relatively high frequency of NA in northern New England and adjacent Canada in 1977, supported the hypothesis that AG had first appeared in the Midwest of the United States and is now spreading eastward (12). The 1977 study also revealed that a small area near Kansas City, KS, contained high proportions of NA. Because the disease appeared in the Kansas City area about 320 km from the nearest known disease center as early as 1952, NA probably represented the original population of *C. ulmi*. A similar situation was found in the town of Millinocket, ME, where the elm population is isolated by forests primarily of spruce and fir. When the disease was first detected there in 1957, the nearest known disease center was about 160 km to the south. In 1977, NA predominated in Millinocket.

In 1980 and 1983, systematic surveys of *C. ulmi* were made to monitor the spread and possible increase of AG in northeastern North America. The surveys were conducted in the state of Vermont and in the town of Millinocket, ME, where high proportions of the *C. ulmi* population were found to be NA in 1977. This paper presents the results of those surveys. A preliminary report of the 1980 survey was published (13).

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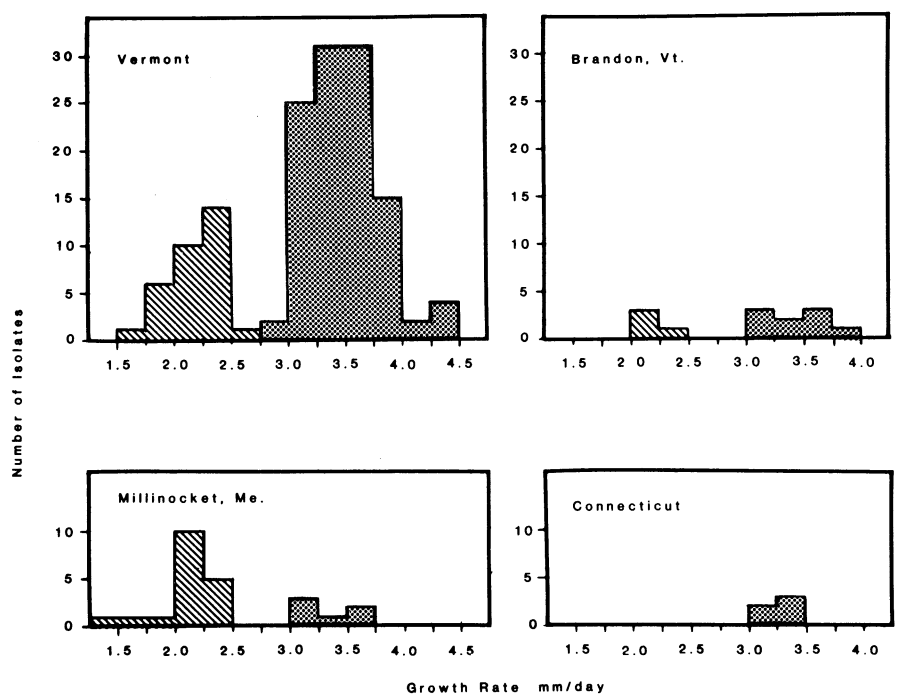

Fig. 1. Growth-rate distribution of 1980 isolates of *Ceratocystis ulmi* on 2% Oxoid malt extract agar at 20 C. Isolates with growth rate and morphology of the aggressive strain (right) are indicated by the dot pattern (142 isolates collected from Vermont; 24 from Millinocket, ME; 13 from Brandon, VT; and 5 from Connecticut).

MATERIALS AND METHODS

Sample locations were determined by a 10,000-m grid that was based on the transverse mercator system. Five or more diseased trees, located as close to the grid intersections as possible, were sampled. In 1980, sampling was concentrated in areas shown to have high proportions of NA in 1977. In 1983, sampling was done in those same areas plus others shown by the 1980 survey to also have significant populations of NA.

Branch segments 12–18 cm long were cut from diseased branches, placed on ice while in the field, and refrigerated until isolation. Most samples were collected during 1-wk periods during mid-August of 1980 and 1983. Sampled trees (at least 45 m apart to avoid the possibility of root-graft transmission) represented a wide range of size and disease conditions—from small saplings to large mature field and roadside trees and from trees with initial wilt symptoms to trees nearly dead.

Isolations were made within a few weeks of collection, and cultural morphology and growth measurements of isolates on 2% Oxoid malt extract agar were recorded as described by Gibbs et al (11) and Brasier (3). Colony growth was measured along four radii after 2 and 7 days in the dark at 20 C; the mean daily growth rate of three replicate plates was calculated for the 5-day period. The cultures were then grown under normal daylight for 14 days before being examined for cultural morphology. In 1983, but not in 1980, isolates whose growth rates at 20 C were in the range where overlap between the two strains was possible also were grown at 31 C, because the two strains show a reversal in growth rates when grown at these two temperatures (8).

In 1980, single isolates of *C. ulmi* from 142 trees in Vermont and 24 from Millinocket were identified as NA or AG. In addition, isolates from 13 trees in Brandon, VT, and five in southern Connecticut were identified. Brandon was sampled intensively to determine if the proportions of NA and AG isolates from diseased street trees there, which unlike those in Millinocket were not isolated from wild populations of field and roadside elms, differed from those of diseased wild elms. In 1983, single isolates from 225 trees in Vermont and nine in Millinocket were compared. In the 1977 study (11) and in both 1980 and 1983, the sample from Millinocket included all accessible, visibly diseased trees.

RESULTS

Separation of isolates. As in the 1977 study, most of the isolates from Vermont and Millinocket, as well as those from Connecticut, could be readily separated into two groups typical of AG and NA on the basis of growth rate and cultural morphology. The growth-rate distributions of the 1980 and 1983 isolates were plotted (Figs. 1 and 2).

As expected, there was variation in growth rates and patterns and in morphology among isolates of both strains. In the 1980 sample, two isolates from Vermont produced protoperithecia, and seven isolates (one from Maine, one from Connecticut, and five from Vermont) that showed an abnormal dark brown "amoeboid" growth pattern were not classified or included in the measurements. In 1983, although three

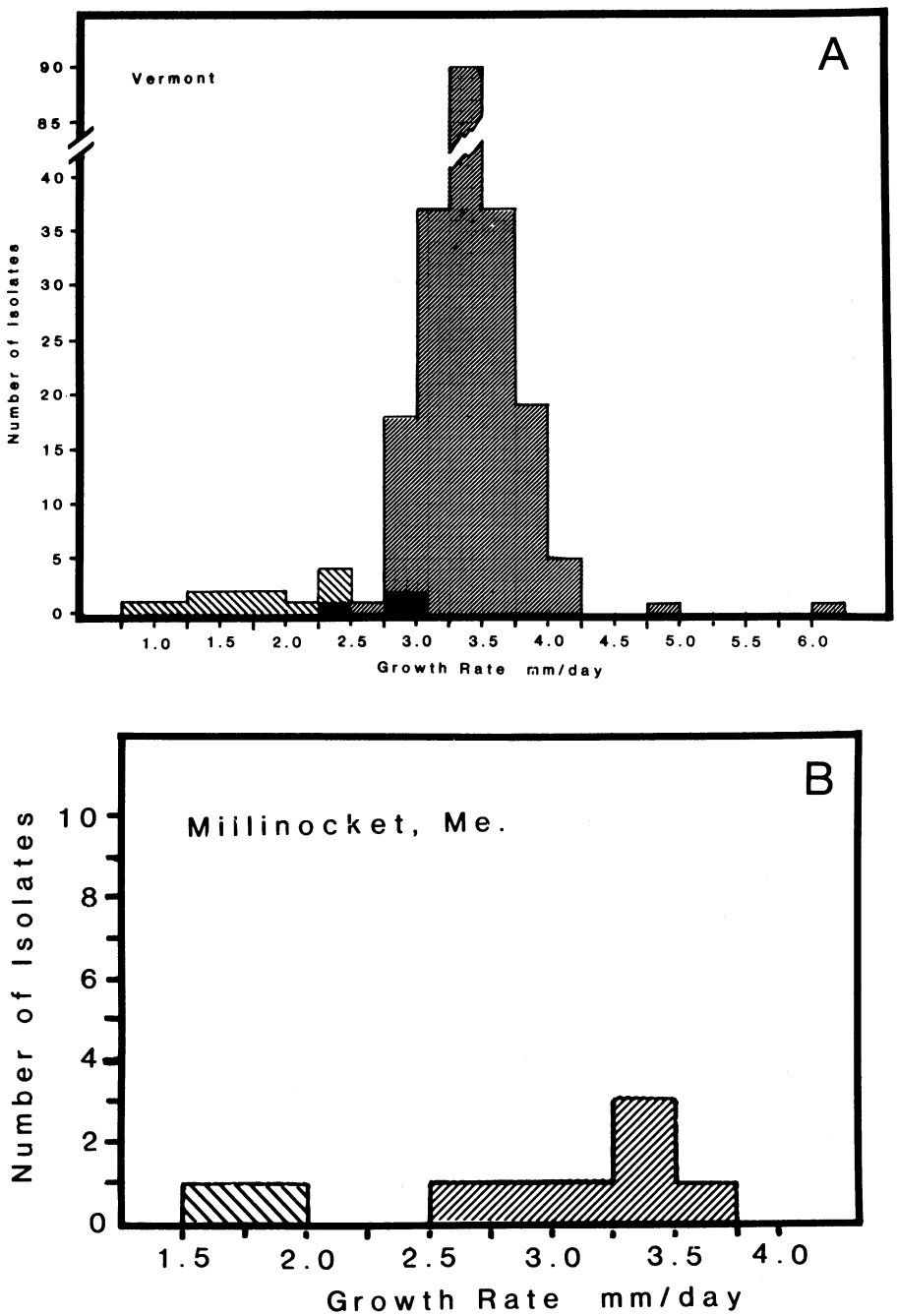

Fig. 2. Growth-rate distribution of 1983 isolates of *Ceratocystis ulmi* on 2% Oxoid malt extract agar at 20 C. Isolates with growth rate and morphology of the aggressive strain (right) are finely hatched. In **(A)** Vermont, 225 isolates were collected; in **(B)** Millinocket, ME, nine isolates were collected.

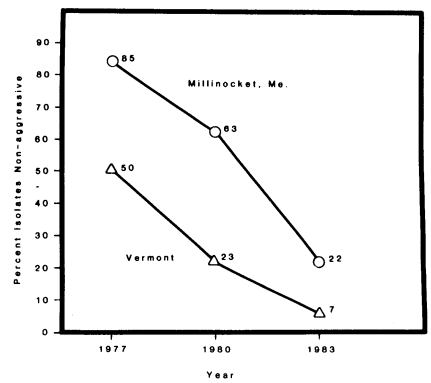

Fig. 3. Decline in proportion of nonaggressive isolates of *Ceratocystis ulmi* in Vermont and Millinocket, ME, from 1977 to 1983.

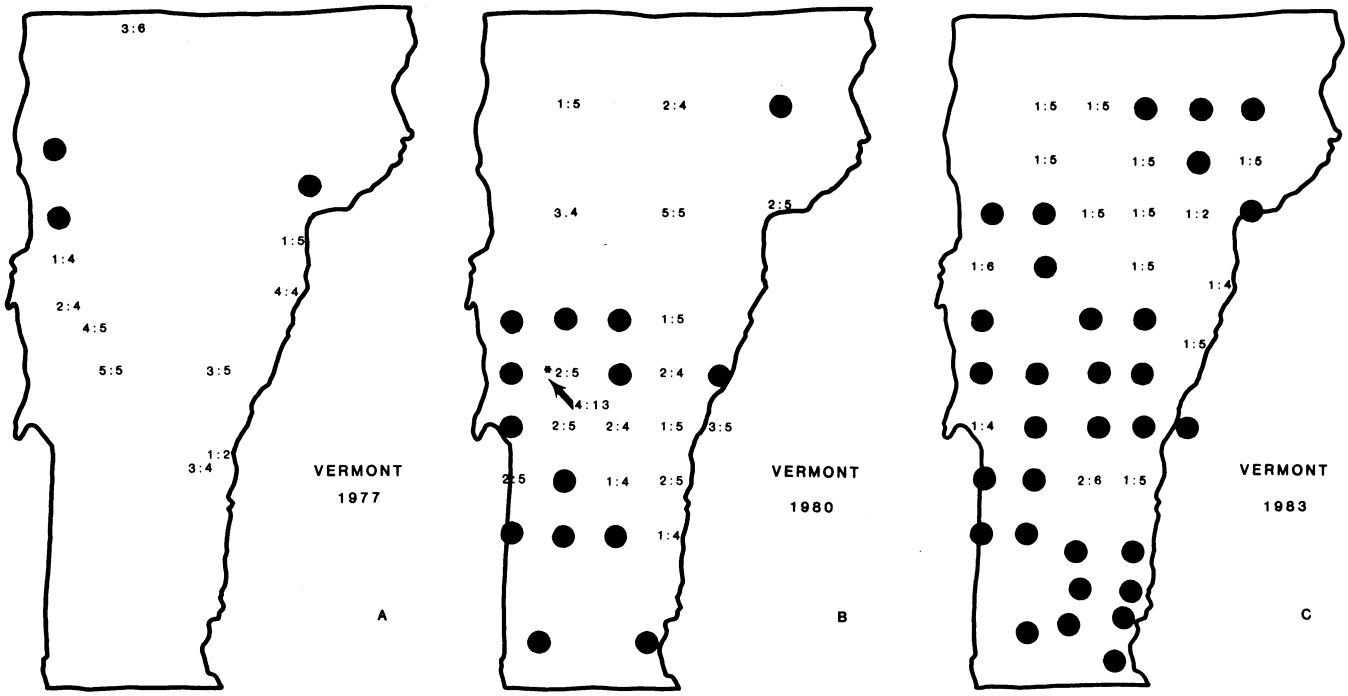

Fig. 4. Distribution patterns of aggressive and nonaggressive strains of *Ceratocystis ulmi* in Vermont in **(A)** 1977, **(B)** 1980, and **(C)** 1983. The ratio of nonaggressive isolates to total isolates at each location is shown. Locations where all isolates were of the aggressive strain are indicated by black spots. (4A adapted from Gibbs et al [11])

isolates could not be placed confidently in either strain, a number of amoeboid-type isolates were classified on the basis of their growth rates at the two temperatures. The growth patterns of these isolates suggested that they might represent "diseased" isolates as described by Brasier (6). This possibility was confirmed when a representative sample of them was examined in 1984 (C. M. Brasier, *personal communication*). Isolates with this growth pattern were distributed widely. They were present in one or more trees at 14 of the 45 sample locations in Vermont and in one of the nine trees from Millinocket. Twenty-two isolates from Vermont produced protoperithecia (12 abundantly).

Distribution of isolates with AG and NA cultural characteristics. Dramatic shifts in the populations of the NA and AG subgroups in Vermont and Millinocket occurred between 1977 and 1983, when the proportion of trees infected with NA in both areas declined from 51 to 7% in Vermont and from 84 to 22% in Millinocket (Fig. 3). The proportions of the isolates from each sample location in Vermont that were NA are shown in Figure 4. On these maps, the numbers represent the ratio of NA to total isolates from that location. The black spots indicate locations where 100% of the isolates were AG. The Brandon, VT, sample location is indicated by the asterisk on the 1980 map. The results of the 1977 survey are provided for comparison (Fig. 4A). Although the numbers of locations sampled were not the same, and although the locations in 1977 were not selected systematically as they were in 1980 and 1983, general trends in the geographic distribution patterns are apparent. The midcentral areas of Vermont, shown in 1977 or 1980 to contain relatively high proportions of NA, had successively lower proportions of this subgroup in succeeding surveys. The percentage of sample locations that yielded one or more NA isolates decreased from 77 in 1977 to 53 in 1980 and 33 in 1983, and the average number of diseased trees per location from which NA type isolates were recovered decreased from 2.1 in 1977 to 1.1 in 1980 and to 0.4 in 1983. In 1980, NA was present in about 30% of the street trees sampled in Brandon (Fig. 4B). This was not significantly different from wild field and roadside elms nearby (40% NA).

DISCUSSION

If the shift to the AG strain of *C. ulmi* continues at the rate shown in this study, the NA subgroup will soon disappear from the areas surveyed. Widespread replacement of NA by AG in North America is indicated by shifts in relative frequencies of the two strains within earlier populations in Maine and by suggested shifts in Massachusetts based on the reduction in the frequency of A mating type (A mating type is rare in AG) (11). Thus, the relationship of the two strains in North America seems to be following a course similar to that reported in Europe. In England, the NA subgroup accounted for more than 22% of the infections in 1971, but by 1978 it had decreased to less than 1% (5). Today, only a few years after the eruption of the second Dutch elm disease epidemic in the late 1960s, NA appears to be virtually extinct in southern Britain (C. M. Brasier, *personal communication*). The slopes of the sharp declines of NA in Britain and in Holland (5) closely match those reported here. The findings in this study, that very few isolates could not be classified confidently as either NA or AG, are supported by earlier evidence from Britain showing that even though the two strains can be in physical proximity to each other in nature and can be forced to hybridize in the laboratory, hybridization between them in nature is virtually nonexistent (1,4,7).

The seeming persistence of NA in certain areas of Vermont suggests that mountainous areas may provide a fleeting stronghold for this strain in North America. Because elms do not grow at high elevations, mountain ranges may present barriers to beetles carrying AG into the area. The patterns of distribution change suggest that such barriers are only temporary and that once they are breached or flanked, AG can spread rapidly through the elm-rich valleys. The reduction in numbers of the longstanding NA and the corresponding increase in AG in Millinocket demonstrate how rapidly this shift can occur once AG has been introduced into an isolated elm population.

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