Changes in Nonaggressive and Aggressive Subgroups of *Ophiostoma ulmi* Within Two Populations of American Elm in New England

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ABSTRACT

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Surveys of elms with Dutch elm disease in Vermont (1989) and in Millinocket, ME (1986 and 1989), revealed the continued decline of the nonaggressive (NA) subgroup of *Ophiostoma ulmi*. This subgroup has virtually disappeared from Vermont; only two of 200 isolates were NA. The same trend was apparent for the isolated elm population in Millinocket, where less than 10% of the 1989 isolates were NA. The pattern of spread of the aggressive (AG) subgroup within Vermont appears to support the concept that this subgroup entered the state from the west and south along elm-rich valley corridors. Since 1980, the structure of the diseased elm population has shifted from scattered, large trees to abundant, small trees and saplings.

Previous surveys of *Ulmus americana* L. with Dutch elm disease have documented that in northeastern North America, the proportion of trees infected with the AG subgroup of the Dutch elm disease pathogen, *Ophiostoma ulmi* (Buisman) Nannf. was increasing sharply compared with the NA subgroup (4,5). On the basis of systematic surveys conducted in Vermont and in Millinocket, ME, in 1977, 1980, and 1983, Houston (5) speculated that if the rate of decrease of the NA subgroup continued, this previously established subgroup would soon be replaced by the AG subgroup in these areas.

This paper reports the results of surveys conducted in 1986 (Millinocket) and 1989 (Millinocket and Vermont) to track any additional changes in pathogen populations. Because the NA subgroup is now virtually extinct in many areas of North America and Europe (5), an ancillary purpose was to obtain as many NA isolates as possible for future phylogenetic studies.

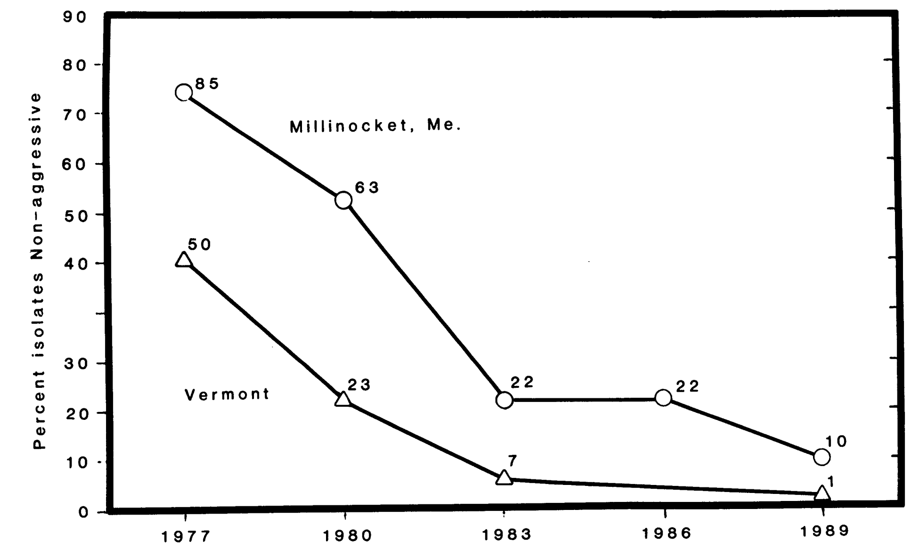

Fig. 1. Decline in proportion of nonaggressive isolates of *Ophiostoma ulmi* in Vermont and Millinocket, ME, 1977–1989.

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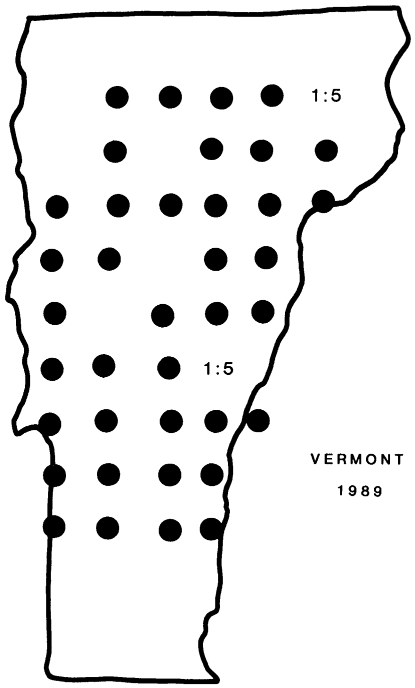

Fig. 2. Distribution pattern of aggressive and nonaggressive subgroups of *Ophiostoma ulmi* in Vermont in 1989. The ratio of nonaggressive isolates to total isolates for the two locations where nonaggressive isolates were captured is shown. Locations yielding only aggressive isolates are indicated by black dots.

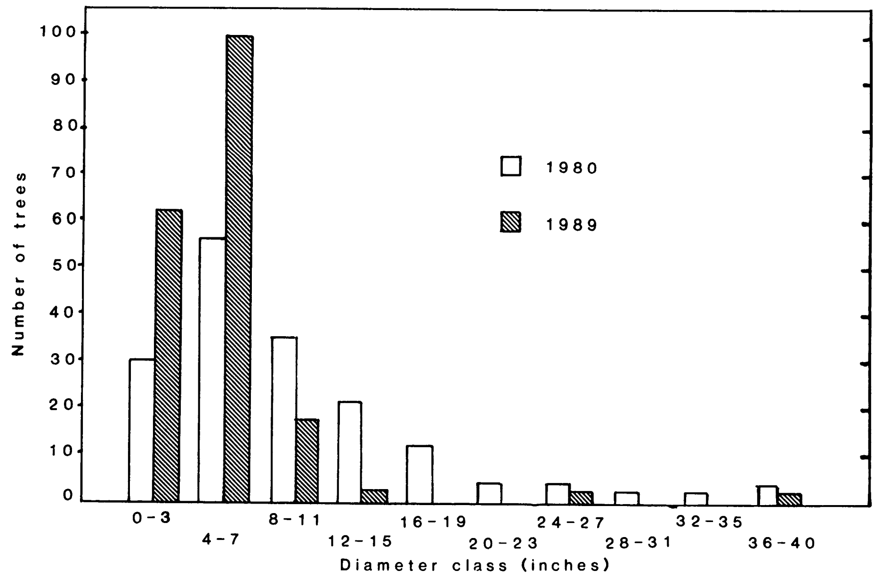

Fig. 3. Size-class distribution of diseased elm populations sampled in Vermont in 1980 and 1989.

MATERIALS AND METHODS

Vermont. In August 1989, 40 locations, chosen by use of a 10,000-m grid based on the transverse mercator system, were visited and twig samples were collected from the five nearest symptomatic elms. All locations yielding NA isolates in 1983 were included in the sample matrix. The 200 trees sampled represented a wide range of sizes, locations, and disease conditions. Trees were at least 45 m apart to reduce the possibility that sampled trees were connected to each other by root grafts. Five to six twig sections, 12–18 cm long, were collected from each tree, stored on ice in separate polyethylene bags during the week collection period, and refrigerated at 4 C until isolation within 2 wk. Isolations were made by placing thin slices of tissue cut from twigs that had been debarked and flamed briefly onto 2% malt agar. Isolates were stored on 2% malt agar at 4 C until growth trials in early December.

Millinocket. All accessible symptomatic trees in this northern Maine town were sampled in July 1986 (27 trees) and in August 1989 (32 trees). Isolates recovered in 1986 were classified to subgroup by C. M. Brasier, Forestry Commission, Farnham, England.

Isolates were classified as NA or AG on the basis of their growth rates and cultural morphology on 2% Oxoid malt extract agar, as described by Gibbs et al (4), Brasier (1), and Houston (5). Isolates for which the average daily growth rates at 20 C were in the range where

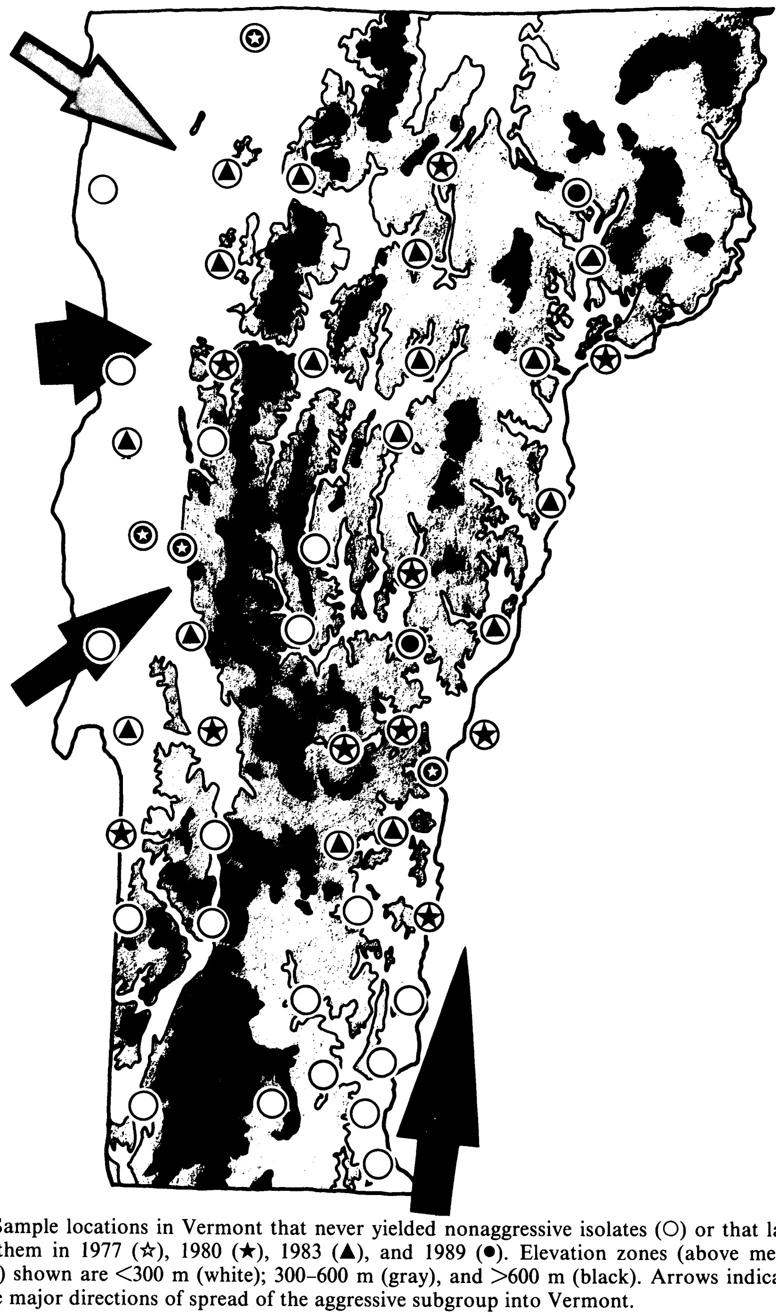

Fig. 4. Sample locations in Vermont that never yielded nonaggressive isolates (○) or that last yielded them in 1977 (☆), 1980 (★), 1983 (▲), and 1989 (●). Elevation zones (above mean sea level) shown are <300 m (white); 300–600 m (gray), and >600 m (black). Arrows indicate probable major directions of spread of the aggressive subgroup into Vermont.

overlap between subgroups was possible were also grown at 32 C. Relative growth rates of the two subgroups are reversed at these two temperatures (3).

RESULTS AND DISCUSSION

Distribution of subgroups. As in previous surveys, nearly all of the isolates from the two elm populations were readily classified as belonging to the AG or NA group.

The NA subgroup has all but disappeared from the surveyed area in Vermont. Only 2 (1%) of the 200 trees sampled yielded an NA isolate (Fig. 1). The two trees were from widely scattered eastern and northeastern locations where trees sampled in 1983 did not yield the NA strain (5) (Fig. 2). The proportion of sample locations that yielded one or more NA isolates decreased from 77% in 1977 to 53% in 1980 and from 33% in 1983 to 5% in 1989; the average number of diseased trees per location that yielded NA isolates decreased from 2.1 in 1977 to 1.2 in 1980 and from 0.4 in 1983 to 0.1 in 1989.

Of the 27 isolates from Millinocket in 1986, six (22.2%) were classified as NA (Fig. 1) (C. M. Brasier, *personal communication*), whereas in 1989, three of 31 isolates (9.7%) were NA (Fig. 1). One isolate from Millinocket could not be classified because of a high level of cultural instability.

Thus, between 1977 and 1983 in Vermont, the proportion of isolates that belonged to the NA subgroup declined an average of 7.2% annually; between 1983 and 1989 they declined 0.97% annually. For the 12-yr period, the value was an average annual decline of 4.0%. Comparable values for Millinocket are 10.5, 2.1, and 6.3, respectively. These decline rates are similar to those encountered in England where 22% of isolates were NA in 1971 compared with less than 1% in 1978 (2), an average annual decline of 3%.

Nature of the elm population. An impression gained during successive surveys was that the population of elms exhibiting active wilt symptoms is shifting from scattered, large trees to abundant, smaller ones. The shift in population structure from large to small trees can be seen in Figure 3. In 1980, about half of the sampled trees were less than 20 cm in diameter; in 1989, nearly 90% were in this category. Conversely, in 1989, only 2.5% of the sampled trees were 30 cm in diameter or larger, compared with 27% in 1980. This shift probably reflects not only the inexorable mortality of the bigger trees but also a concomitant increase in the rate of infection of smaller trees and saplings. The rapid loss from the population of most of the large trees, which comprise not only the main source of elm seed but also the most abundant and favorable brood sites of the beetle vectors, may set the stage for future oscillations in disease intensity.

Patterns of spread of the aggressive subgroup in Vermont. The pattern of decline of the NA subgroup and concomitant increase of the AG subgroup occurred from west to east and from south to north (Fig. 4). These patterns support the hypothesis that the AG subgroup has spread eastward from the Midwest of the United States into regions previously occupied by the NA subgroup (4,5).

Other Eastern mountainous areas may provide temporary refuge for the NA subgroup. Mid-Atlantic Appalachian states are likely candidates. Indeed, an NA isolate was recovered from a valley elm in northeastern West Virginia in 1983 (D. R. Houston, *unpublished*). Should additional NA isolates be needed for future genetic studies, it is probable that they will be found, if at all, only in such locations.

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