Aggressive and Non-aggressive Strains of *Ceratocystis ulmi* in North America

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ABSTRACT

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Examination of the culture morphologies and growth rates of 300 isolates of *Ceratocytis ulmi* collected during 1977 from a range of locations in eastern and central North America revealed that all but one could readily be assigned to the two strains ("aggressive" and "non-aggressive") originally defined in Britain. A sample of 70 isolates collected in 1970 and maintained on autoclaved elm twigs at −20 C also was classified similarly. In the North Central states of the USA the aggressive strain was predominant, but in northern New England and the adjacent provinces of Canada, and in a small sample from Kansas, the non-aggressive strain was detected more frequently. Available evidence indicates that the aggressive strain is migrating into the northeast and this phenomenon is discussed in relation to current theories on the origins of the two strains of *C. ulmi*.

Research in Britain on the devastating epidemic of Dutch elm disease which erupted in the late 1960s showd that the causal fungus, *Ceratocystis ulmi* (Buis.) Moreau, existed as two strains, called "aggressive" and "non-aggressive" on the basis of their pathogenicity to *Ulmus procera*. On 2% Oxoid malt extract agar the two strains differed in growth rate and culture morphology (5).

At the same time, it was found that the aggressive strain also was present in North America (5) and that it probably reached Britain in shipments of rock elm, *Ulmus thomasii*, from Canada (1). Subsequent inoculation experiments showed that the non-aggressive strain also was present in the USA (6), but there has been some doubt as to whether all North American isolates of the fungus could be classified in the two strains (6,16).

To study the situation further, isolates of *C. ulmi* were obtained from diseased elms in many different parts of North America during 1977 and their cultural characteristics were examined. Most of the samples came from New York, New England, and the adjacent provinces of Canada, although 50 isolates were obtained from Minnesota. In addition, we examined 70 isolates collected in 1970 from within the United States, and stored frozen since then at Madison, Wisconsin, on autoclaved elm twigs. These isolates principally were from Wisconsin and other North Central states, although some were from New England and Kansas.

MATERIALS AND METHODS

In 1977, the U.S. samples were collected between early June and early September. At any one location, diseased twigs were obtained from 5–10 trees spaced sufficiently far apart that root transmission of the fungus from one tree to another was unlikely. In addition, the following people kindly supplied freshly collected samples: R. J. Campana (Orono, Maine); F. W. Holmes (Massachusetts); L. S. Magasi (the Maritimes); G. B. Ouellette (Quebec); and R. Ullrich (Burlington, Vermont). The samples were refrigerated for as much of the time between collection and isolation as possible, and cultures were examined within a few weeks of isolation.

In 1970, a collection of US isolates was assembled by one of us (E. B. Smalley). Among the chief contributors were R. J. Campana (Orono, Maine); D. W. French (Minnesota); F. W. Holmes (Massachusetts); F. L. Howard (Kingston, Rhode Island); D. Neely (Illinois); H. E. Thompson (Manhattan, KS); and the State Department of Agriculture in Wisconsin. The isolates were individually transferred to autoclaved elm twigs in a glass vial,

incubated, and (after coremium production was well advanced) stored at −20 C. In November, 1977, the fungus was successfully re-isolated from most of the vials.

Cultural characteristics were compared as described by Gibbs and Brasier (5). Two percent Oxoid malt extract agar (2%) (K. C. Biological Inc., P.O. Box 5441, Lenexa, KS 66215) was prepared by dissolving 33 g of Oxoid malt extract agar plus 10 g of agar in 1L of distilled water and autoclaving the mixture for 10 min at 1.05 kg/cm^2 pressure. The growth rate experiments were carried out in the dark at 20 C with two colony diameters being measured after 2 days and again after 7 days. The mean daily radial growth rate then was calculated for the 5-day period. Three replicate plates were prepared for each isolate. Standard isolates from England, W7 (non-aggressive) and RDT2 (aggressive), were used as controls. After the second growth measurement, the cultures were kept on the laboratory bench for 2 wk before being examined for colony morphology.

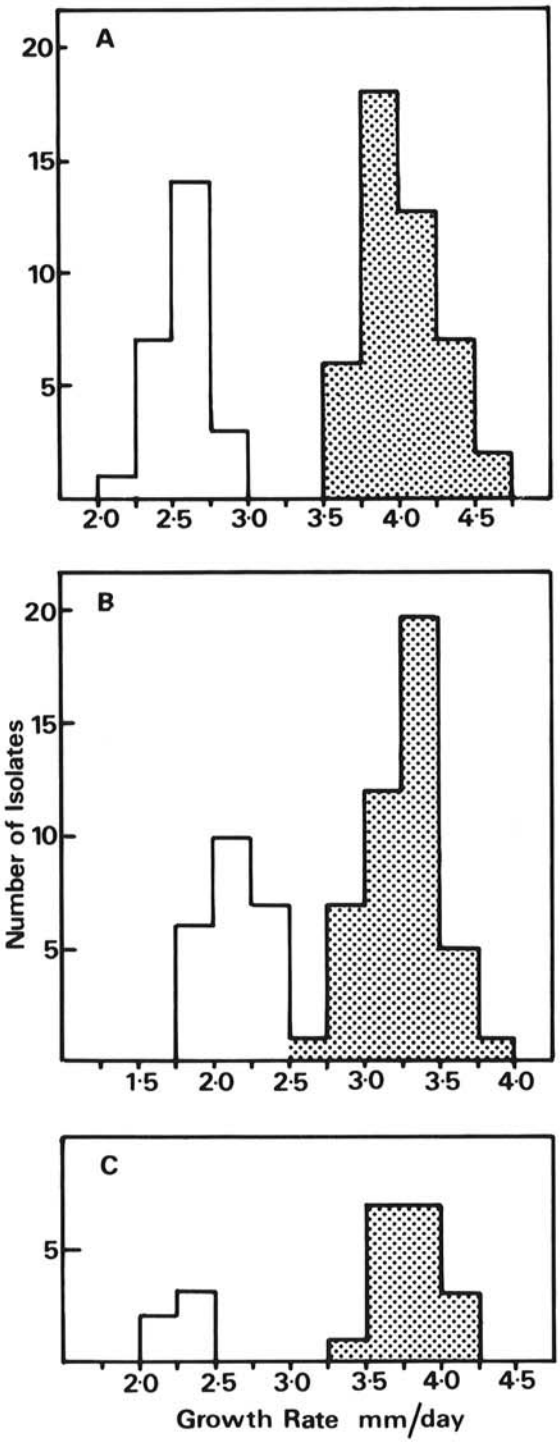

Fig. 1. Growth rate distribution of various samples of *Ceratocystis ulmi* on 2% Oxoid malt extract agar at 20 C; isolates with 'aggressive' morphology are stippled. **A,** 69 isolates collected in 1977 from Maine. **B,** 64 isolates collected in 1977 from a range of locations in North America. **C,** 25 U.S. isolates collected in 1970 and maintained on autoclaved elm twigs at −20 C.

RESULTS

Cultural characteristics of the isolates. It is not possible for all the growth rate data on all the isolates to be presented in full. Moreover, detailed comparisons between data from different growth runs could not be made because of the effects on growth rate of slight variations in incubator temperature and the age of the agar plates.

Representative data on 69 isolates from Maine are shown in Fig. 1-A. On the basis of growth rate, two quite distinct groups

TABLE 1. Distribution of the two strains (aggressive and non-aggressive) of *Ceratocystis ulmi* in North America

Location	Collected	Non-aggressive (no.)	Aggressive (no.)	Non-aggressive (%)
Canadian Provinces:				
New Brunswick	1977	9	6	60
Nova Scotia	1977	3	2	60
Quebec	1977	5	1	83
U.S. Northeastern states:				
Connecticut	1970	0	1	0
	1977	0	6	0
Maine	1970	4	9	31
	1977	23	59	28
Massachusetts	1970	1	5	17
	1977	0	22	0
New Hampshire	1977	6	9	40
New York[a]	1970	0	3	0
	1977	3	36	8
Rhode Island	1970	0	6	0
Vermont	1977	27	28	49
U.S. Northcentral states:				
Illinois	1970	0	6	0
Minnesota	1970	1	6	14
	1977	0	50	0
Wisconsin	1970	0	16	0
U.S. Plains state:				
Kansas	1970	8	4	66

[a]The New York sample also included one isolate which could not be classified (see text).

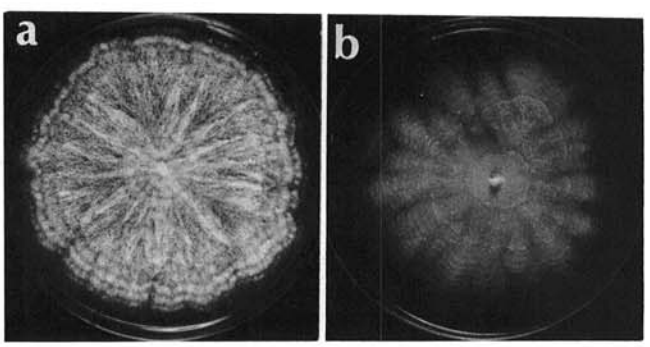

Fig. 2. Cultural morphology of representative North American **a,** aggressive and **b,** non-aggressive isolates of *Ceratocystis ulmi* on 2% Oxoid malt extract agar.

existed. Isolates in the fast-growing group possessed the typical fibrous striate colony morphology of the aggressive strain; the slow-growing isolates had little aerial mycelium and possessed the 'waxy' appearance of the non-aggressive strain (2,5). Representative cultures are illustrated in Fig. 2.

Isolates from elsewhere also could be classified into the two strains. This is illustrated in Fig. 1-B which comprises data on isolates from 64 locations from Minnesota in the west to Nova Scotia in the east. In growth rate the two groups were not so widely separated as in the Maine sample, but by using colony morphology as a character, all the isolates could be classified readily into one strain or the other. Statistical analysis of the data showed that, in addition to the obvious highly significant difference between the two strains, there were significant differences within each strain (Neuman-Keuls multiple range test, $P=0.05$). We hope to conduct pathogenicity studies on fast- and slow-growing isolates within the aggressive strain at some future date.

In all, 300 isolates from 1977 were examined. Of these, 299 could confidently be assigned to one or the other of the two strains. The remaining isolate, NY11 from Lyons Falls, New York, possessed a morphology typical of the aggressive strain but had a growth rate only a little above the mean of the non-aggressive strain. Also, four aggressive isolates (two from Maine and two from Minnesota) produced 'protoperithecia' and thus were suspected of belonging to the rare mating type A of the fungus (3). This was confirmed when, upon pairing on malt agar with a known B isolate, perithecia were produced. A random sample of 30 other aggressive isolates proved, as expected, to be of the B mating type.

Among 25 isolates collected in 1970 from New York and New England, the clear separation of the groups again was apparent (Fig. 1-C). For four of the isolates, pathogenicity and growth rate data already were available from the work of Gkinis (7); our results confirmed her data.

It recently has been shown that the two strains of *C. ulmi* have different temperature/growth rate relationships (12 and J. Lea and C. M. Brasier, *unpublished*). On 2% Oxoid malt extract agar, aggressive isolates grew best at 22–23 C and non-aggressive isolates grew best at 28–30 C. At 33 C, the growth rate of the non-aggressive strain was significantly higher than that of the aggressive strain. A sample of 11 North American isolates of each strain from 1977 behaved similarly. All the non-aggressive isolates grew more rapidly at 30 C than at 20 C, whereas all the aggressive isolates grew

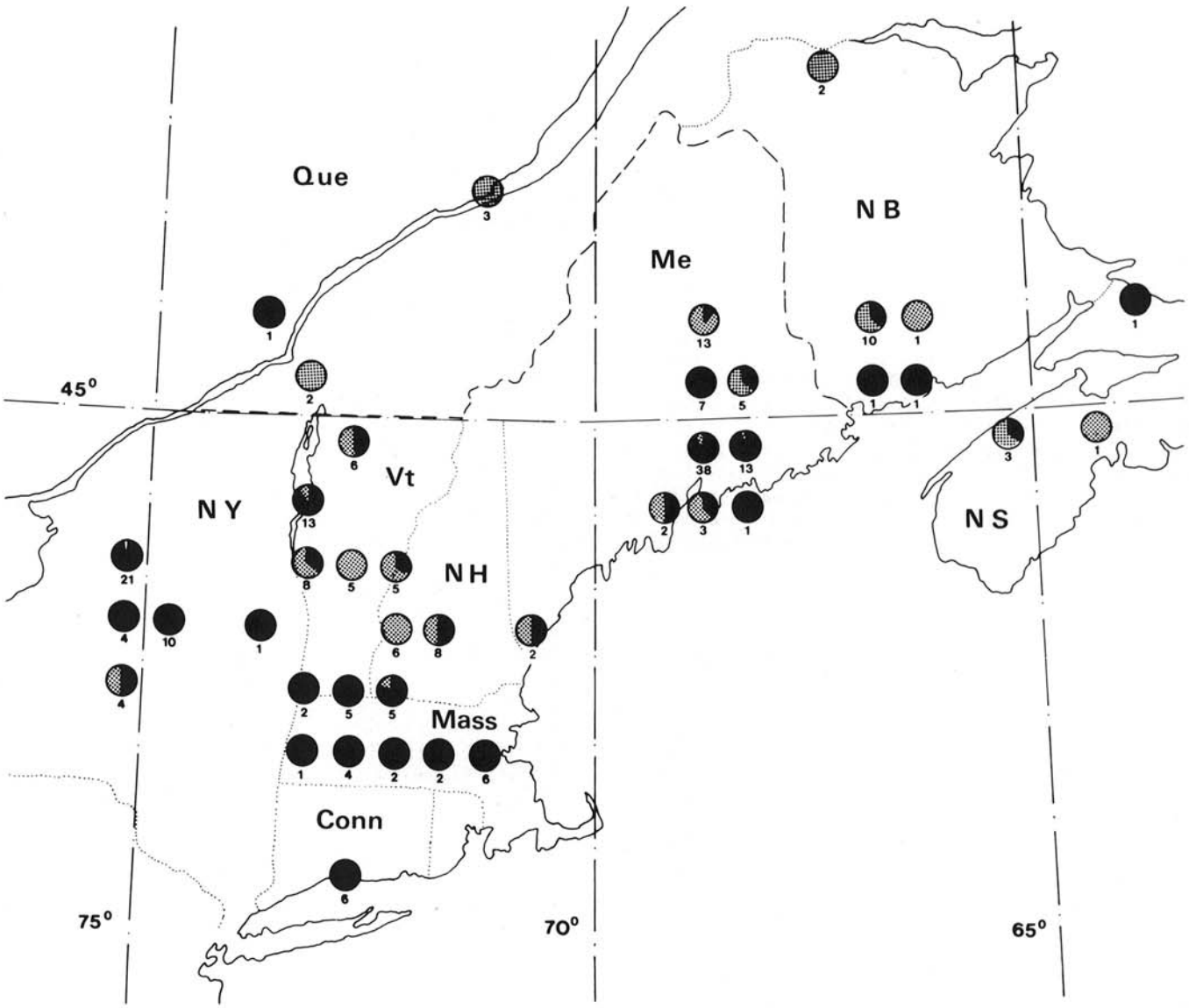

Fig. 3. Distribution of aggressive and non-aggressive strains of *Ceratocystis ulmi* in northeastern North America. Each circle represents a unit area 0.5° longitude × 0.5° latitude and the figure below it shows the number of *C. ulmi* isolates obtained from that unit area. The circle is sectored to illustrate the relative proportion of aggressive isolates (black) and non-aggressive isolates (stippled). State or province boundaries are shown by dotted lines and the U.S.-Canadian border by a dashed line.

more slowly at 30 C than 20 C. Between these two temperatures the average increase in the growth rate of the non-aggressive isolates was 52% and the average decrease in the growth rate of the aggressive isolates was 30%.

Geographical distribution of the strains. Table 1 shows proportions of the two strains among isolates grouped by state (USA) or by province (Canada). There are quite large differences in the incidence of the non-aggressive strain. In the North Central states of Minnesota, Wisconsin, and Illinois the non-aggressive strain is very rare. This is a similar situation to that reported in Iowa by McNabb (14) who found that of 500 isolates examined in 1973 only two or three were non-aggressive. In marked contrast, two thirds of the isolates were non-aggressive in the 1970 sample from Kansas (Table 1).

The aggressive strain predominates in New York, Connecticut, Rhode Island, and Massachusetts but in the northern New England States of Vermont, New Hampshire, and Maine, the non-aggressive strain is a significant proportion of the total. In the neighboring Canadian provinces of Quebec, Nova Scotia, and New Brunswick the latter strain is in the majority. This distribution is illustrated for the 1977 isolates in Fig. 3. In the Northern Eastern area, where the non-aggressive strain is relatively common, there appeared to be a relationship between its frequency in the population and the distance from the Atlantic Ocean. Thus, in Maine sampling was conducted in relation to the recent intensification of the disease in the coastal towns from Belfast to Ellsworth, and on a transect inland from the coast through Orono and Lincoln to Millinocket near Baxter State Park, where the disease was detected as early as 1957 but where the bulk of the elm population remained. In the coastal samples the aggressive strain predominated but at Lincoln 3 out of 5 isolates were non-aggressive and at Millinocket 13 out of 15. A similar situation was found in New Brunswick. In Maine and New Brunswick together the non-aggressive strain made up only 15% of the 59 isolates collected within 56.3 km of the Atlantic, as compared to 61% of the 38 isolates from further away.

DISCUSSION

It is now evident that the vast majority of fresh *C. ulmi* isolates from North America can be classified into two strains, aggressive and non-aggressive. Moreover, the cultural characteristics of the wild types remained stable during storage on elm wood at − 20 C. Difficulties encountered in earlier work probably were due principally to changes in the cultures during their maintenance on conventional laboratory media. This already has been suggested for some American isolates (6), and might explain (at least in part) the results of Schreiber and Townsend (16) who worked with some isolates that had been held in culture for at least 3 yr. Other factors important in strain differentiation are the incubation temperature (12) and the type of agar used. Thus, the 'waxiness' of the non-aggressive strain on 2% Oxoid malt extract agar is not present in cultures on Difco malt extract agar. Even on the Oxoid medium there may be variation between one batch of cultures and another, particularly if they have been stored for different periods prior to use. Factors of this kind probably account for the data obtained by Gkinis (7) at Wisconsin with 10 of the isolates from the 1970 collection. She found that these isolates fell into two groups on the basis of growth rate and pathogenicity, but was unable to differentiate between them in terms of culture morphology. Because of these various complications, the defined agar medium recently developed by Hindal and MacDonald (8) may prove a useful additional aid to strain identification.

The distribution of the two strains. This paper represents the first attempt to characterize the *C. ulmi* population of North America in terms of the two strains. Much remains to be done, but nevertheless some general conclusions can be drawn. First, there is a marked difference between the eastern part of the continent where the non-aggressive strain is common, and North Central U.S. where it is very rare. Second, the sample from Kansas suggests that there may well be other places far removed from the North Eastern area where the non-aggressive strain is an important component of the fungus population.

There has been some speculation about the origins of the two strains (4,5,13). Initially Gibbs and Brasier (5) suggested that both strains were present in Europe in the 1920s and that both reached North America in the 1930s. Subsequently, however, McNabb (13) has postulated that initial spread of the disease on both continents involved only the non-aggressive strain and that the aggressive strain appeared somewhere in the North Central states of the USA, perhaps Illinois, in the 1940s. The aggressive strain, which possesses slightly greater pathogenicity even to the highly susceptible *U. americana*, is then thought to have spread eastward across the continent killing elms that had escaped infection by the non-aggressive strain. In the process, it was introduced to Europe. The relatively low frequency of the aggressive strain in the North East and its association with areas of recent disease intensification support McNabb's hypothesis (13). Comparison of the 1970 and 1977 data from Orono, Maine, also supports the same hypothesis. Thus, the aggressive strain made up only 71% of the 1970 isolates (9 of 13) as compared to 100% of the 26 isolates examined in 1977. Evidence that a change has occurred in the fungus population also comes from data on mating type. Twenty years ago Holmes (9) reported that cultures from 106 of 112 towns in Massachusetts were of the A mating type. By contrast, all 26 of our 1977 isolates from this state were of the B mating type Because the A mating type is rare in the aggressive strain it seems very probable that this change involved the replacement of the non-aggressive by the aggressive strain. In Wisconsin in 1959 the B mating type comprised 13 of 16 isolates of the fungus (F. W. Holmes, *personal communication*) and this is consistent with the view that the aggressive strain was present in the North Central states much earlier than in the east.

The high proportion of the non-aggressive strain in the 1970 sample from eastern Kansas is of great interest in that although the disease was not recorded until 1957, when a few diseased trees were found in Kansas City (15), it was apparently present as early as 1952 just across the state line in Kansas City, Missouri. In 1957, the nearest known disease foci were some 200 miles to the east near the Illinois border (10). It thus seems possible that the non-aggressive strain represents the initial *C. ulmi* population of the area and the aggressive strain is a more recent arrival from the North Central states.

For a further examination of McNabb's theory (13), studies of the *C. ulmi* population could be made in other areas where early cases of the disease were recorded. Examples would include Tennessee and Virginia (10).

With the hypothesis of Gibbs and Brasier (5) one would have to assume that differences in various areas represented adaptation to local environmental conditions. No basis for such adaptation is known at present. The high temperature optimum of the non-aggressive strain (12) might favor it in Kansas, but could hardly confer the same advantage in Maine, New Brunswick, or Quebec.

Whatever the precise history of the strains, there is clearly a need for further information on strain distribution in North America, particularly in places where substantial populations of healthy elms remain. Nine isolates from Colorado examined in 1973 all were aggressive (C. M. Brasier, *personal communication*) and it has recently been shown that the aggressive strain is well established in California (11). In Washington DC where many important and historic elms remain, a sample supplied in 1978 by J. L. Sherald of the National Parks Service comprised 46 aggressive and five non-aggressive isolates. Further data of this kind are required.

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