Detection of Double-Stranded RNA in *Ceratocystis ulmi*

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ABSTRACT

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Double-stranded RNA (dsRNA) was detected in United States isolates of *Ceratocystis ulmi* by serology and polyacrylamide gel electrophoresis. Molecular weights of dsRNA segments ranged from about 0.4 to 2.0×10^6 daltons. DsRNA segments were present in both more aggressive and less aggressive isolates; however, less aggressive isolates generally possessed more segments. The dsRNA segments that were unique to less aggressive isolates may contribute to diminished pathogenicity, although other unknown factors apparently influence pathogenicity as well. Single-conidial isolates from one less aggressive strain were of three types based on their dsRNA content and cultural characteristics. One segregant type, which contained all seven segments present in the parent strain, was less pathogenic than a second type containing only one segment or a third type having no dsRNA. Whether the dsRNA is of viral origin was not determined. Attempts to detect viruslike particles in semipurified preparations failed.

Additional key words: cytoplasmic inheritance, Dutch elm disease, fungal virus, mycovirus.

It has become apparent in recent years that viral infections are not uncommon in fungi. Well-documented examples in which viruses or dsRNA have been associated with altered fungal phenotypes include the killer systems in *Saccharomyces cerevisiae* (2) and *Ustilago maydis* (24), and the 'La France' disease of *Agaricus bisporus* (34). Viruses or viruslike particles (VLPs) have been reported in over 30 species of plant pathogenic fungi (24). In several cases, the effects of the virus on pathogenicity of the fungus have been studied. Viruses seem to play little or no part in determining pathogenicity of *Gaeumannomyces graminis, Periconia circinata, Colletotrichum lindemuthianum, Fusarium culmorum, Puccinia graminis,* and *Sclerotium cepivorum* (21). In these studies, however, comparisons were possible only between naturally occurring isolates with or without the viruses; until defined strains of the fungus can be experimentally infected, such comparisons are not conclusive.

On the other hand, some fungi contain agents, possibly viruses, that are transmissible by hyphal anastomosis, induce poor growth in culture, and diminish pathogenicity. Examples are *Helminthosporum victoriae* (21), *Pestalozzia* sp. (21), *Podospora anserina* (21), *Endothia parasitica* (17,18), and *Rhizoctonia solani* (7,8). For *E. parasitica* (11) and *R. solani* (9) the decrease in growth rate and virulence is associated with the presence of dsRNA. Furthermore, the dsRNA in *E. parasitica* has been associated with club-shaped VLPs in at least one strain (12).

The term "hypovirulence" has been used to denote suppression of virulence in fungi by cytoplasmically transmissible factors (16,33). Use of hypovirulent strains of plant pathogenic fungi in

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disease control is attractive, but few examples have been reported. Hypovirulent strains of *E. parasitica* (causal agent of chestnut blight) can prevent more virulent strains from attacking the host when both coexist in the same canker (16,33). Similarly, the incidence of postemergence damping-off of sugar beets, caused by *R. solani*, is significantly lower when hypovirulent and virulent isolates occur together in the soil (8).

After Gibbs and Brasier (15) described two distinct strains of *Ceratocystis ulmi* in Britain, Atanasoff (1) suggested that the less aggressive strain may be virus-infected and the more aggressive one free of viruses. Viral infection of *C. ulmi* has also been linked to the attenuation of the severe epidemic of Dutch elm disease (DED) in Britain from 1926 to the mid-1930s (5).

Hollings et al (22,23) examined two British isolates of *C. ulmi*, one more aggressive and one less aggressive, for VLPs and dsRNA. No VLPs were found by electron microscopy in preparations designed to isolate viruses, nor was dsRNA detected in serological tests with antisera to poly I: poly C. Brasier and Gibbs (4,6) found that single ascospore progeny from crosses between more aggressive and less aggressive strains of *C. ulmi* were skewed toward low pathogenicity and none approached the more aggressive parent strain in pathogenicity. Their results could be explained by transmission of viruses that reduce pathogenicity. Nevertheless, they concluded that pathogenicity in *C. ulmi* is under polygenic control and that cytoplasmic factors are not directly involved. To determine whether or not viruses might be related to pathogenicity, United States isolates of *C. ulmi* of known pathogenicity were assayed for dsRNA.

MATERIALS AND METHODS

Isolates and cultures. Fifteen United States isolates of *C. ulmi* were obtained from L. R. Schreiber, USDA, ARS, Nursery Crops Research Laboratory, Delaware, OH 43015. Pathogenicity of these isolates had been previously determined by Schreiber and Townsend (30). Ten of the isolates were of the "more aggressive" type and the other five were of the "less aggressive" type as defined by Hindal et al (19,20). Mycovirus-containing cultures of *Penicillium chrysogenum* Thom (ATCC 9480) and *P. stoloniferum* Thom (ATCC 14586) were used as standards in dsRNA and VLP extractions. All isolates were maintained in screw-top bottles on potato-dextrose agar (PDA) at 5 C.

For mycelia and spore propagation, *C. ulmi* isolates were grown in shake cultures of potato-dextrose broth (PDB) (20 g Difco potato extract; 15 g glucose per liter). Two-liter Erlenmeyer flasks containing 500 ml of PDB were inoculated with 1-cm-diameter agar plugs taken from colonies grown on PDA for 4–7 days at 25 C. The shake cultures were harvested after 8 days of incubation at 25–26 C by centrifugation at 8,000 *g* for 10 min. Fungal growth was washed once by centrifugation in distilled water. Percent dry weight was determined by weighing small samples of the fungus dried at 100 C for 24 hr or longer. The harvested mycelia and spores were frozen in 25 or 50 g portions at −20 C.

Mycelia of isolates of *Penicillium* were mass produced in still culture on a medium containing 5 g NaCl, 1 g K_2HPO_4, 1 g yeast extract, 25 g Bacto tryptone, 3 g glucose, 3 g soybean meal, 4 ml corn steep liquor, and 3 ml glycerol per liter of water (31). One-liter Erlenmeyer flasks containing 250 ml of medium were inoculated with spore suspensions. Mycelial mats were harvested after 12 days.

Extraction and purification of dsRNA. Isolation and purification of dsRNA directly from mycelia of the 15 *C. ulmi* isolates (Table 1), *P. chrysogenum*, and *P. stoloniferum* were by cellulose chromatography (14) as modified by Morris and Dodds (29). From previously estimated values of percent dry weight for *C. ulmi* samples, amounts of frozen mycelia containing approximately 3 g dry weight (10–30 g wet weight) were combined with water to a total weight of 30 g. This was added to 30 ml of extraction buffer (0.2 M glycine, 0.1 M Na_2HPO_4, 0.6 M NaCl, 1.0% sodium dodecyl sulfate, adjusted to pH 9.5 with 5 M NaOH), 30 ml of redistilled phenol saturated with water, and 30 ml of chloroform-pentanol (25:1, v/v). Aliquots of 30 ml were homogenized for 2 min each in a Braun Model MSK mechanical cell homogenizer (Quigley-Rochester Inc., Rochester, NY 14602) and then combined. The total homogenate was centrifuged for 20 min at 8,000 *g* and 5 C. The upper, aqueous phase was removed and adjusted to 15% (v/v) ethanol by adding 95% ethanol. For each 20 ml of 15% ethanol solution, 0.25 g cellulose powder was added (Whatman CF 11). The mixture was stirred for 10 min and then centrifuged at 8,000 *g* for 10 min. The supernatant was discarded and the cellulose pellet was washed with 110 ml of 15% ethanol-STE (STE: 0.05 M Tris-HCl, 0.001 M Na_2EDTA, 0.1 M NaCl, pH 7.0) and recentrifuged. The wash procedure was done three times. Following the last wash, the cellulose-15% ethanol-STE mixture was poured into a glass chromatography column, 1.5 cm ID × 30 cm, and washed with 250–300 ml of 15% ethanol-STE to further remove all nucleic acids except dsRNA. (Less pure dsRNA preparations for serological testing were washed once by centrifugation prior to packing the column.) The dsRNA was eluted from the cellulose column with 100% STE. Two volumes of cold 95% ethanol were added and the solution was placed at −20 C overnight to precipitate the dsRNA. The precipitate was collected by low-speed centrifugation (12,000 *g*, 10 min, 5 C) and resuspended in buffer solutions suitable for electrophoretic or serological analysis.

Immunodiffusion tests. Antiserum to dsRNA was made against a complex of methylated bovine serum albumin and the synthetic dsRNA polyinosinic: polycytidylic acid (poly I: poly C) as described by Francki and Jackson (13). Preparations from the fungal isolates were tested serologically for dsRNA by agar-gel double diffusion tests following the procedure of Moffitt and Lister (28).

Polyacrylamide gel electrophoresis. Samples were dissolved in buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH adjusted to 7.8 with acetic acid) and electrophoresed for 4.5 hr at 5 mA per g/gel in 2.4% polyacrylamide gels cast in 10 × 6 mm (ID) tubes. Following electrophoresis, gels were stained for 30 min or longer in 1 g of ethidium bromide per milliliter, and then photographed under shortwave UV light (Chromato-transilluminator Model C-61; Ultraviolet Products Inc., San Gabriel, CA 91778) on Polaroid Type 55 film using a Wratten G yellow filter. To confirm the presence of dsRNA in electrophoresis gels, nucleic acid preparations from isolate TN were tested for sensitivity to ribonuclease (RNase) and deoxyribonuclease (DNase). Samples were incubated for 30 min at 37 C with 1.0 μg/ml RNase (bovine pancreatic ribonuclease A: Sigma Chemical Co., St. Louis, MO 63178) in buffer consisting of 0.01 M Tris-HCl and either 0.30 or 0.01 M NaCl, pH 7.2. Other samples were similarly treated with 20 μg/ml DNase (RNase-free deoxyribonuclease; Worthington Biochemical Corp., Freehold, NJ 07728) in 0.01 M

TABLE 1. Detection of dsRNA by serology and polyacrylamide gel electrophoresis (PAGE) in preparations from isolates of *Ceratocystis ulmi*

Isolate[a]	Origin	Pathogenicity[b]	Serology[c]	PAGE[c]
AL	Alabama	M	−	−
CO1	Colorado	M	+	+
IA	Iowa	M	−	−
IL	Illinois	M	+	+
MA1[d]	Massachusetts	M	+	+
ME	Maine	M	+	+
MO	Missouri	M	+	+
ND	North Dakota	M	−	−
VA	Virginia	M	−	−
WI	Wisconsin	M	−	−
CO2	Colorado	L	−	+
MA2	Massachusetts	L	+	+
NC	North Carolina	L	−	−
OH	Ohio	L	+	+
TN	Tennessee	L	+	+

[a] Isolates supplied by L. R. Schreiber.
[b] Pathogenicity is based on inoculation experiments by Schreiber and Townsend (30); M = more aggressive and L = less aggressive.
[c] Detection of dsRNA = +.
[d] This isolate was shown to be less aggressive by Schreiber and Townsend (30), but was more aggressive in subsequent inoculation trials by Hindal and co-workers (19,20).

Tris-HCl, 5 mM $MgCl_2$ buffer, pH 7.4. After treatment, 500 mg/ml bentonite was added and the mixture centrifuged at 12,000 *g* for 10 min to remove nucleases. Samples were then chilled, precipitated with ethanol, centrifuged at low speed, and the pellet dissolved in buffer and electrophoresed.

Extraction and purification of VLPs. Frozen mycelium of less aggressive *C. ulmi* isolate TN (Table 1) was used for extraction and purification of VLPs as described by Wood et al (35), Bozarth et al (3), and Moffitt and Lister (27). Two buffer systems, potassium phosphate buffer at pH 7.0 and Tris-HCl buffer at pH 8.5, were tested with each method. Extractions were also made from isolates MA2 and ND using phosphate buffer and the method of Wood et al (35). *P. chrysogenum* virus was extracted and purified with phosphate buffer using each method. The VLPs in preparations from *P. chrysogenum* were compared with the contents of similar preparations from *C. ulmi.*

After differential centrifugation of the extracts, the final high-speed pellets were resuspended in the appropriate buffer and analyzed by rate-zonal centrifugation in 10–40% sucrose density gradients. Gradients were centrifuged for 2.0–2.5 hr in a Beckman SW 41 rotor (Beckman Instruments, Palo Alto, CA 94304) at 120,000 *g* and 5 C, scanned at 254 nm, and fractionated with an ISCO density gradient fractionator (ISCO Instrumentation Specialists Co., Lincoln, NE 68505).

Gradient fractions showing high absorbance were collected for study by electron microscopy. The fractions were diluted with buffer and pelleted at 125,000 *g* for 70 min. Pellets were suspended in 1% phosphotungstic acid, and flooded onto 74-μm (200-mesh) copper grids. All specimens were examined on a Phillips EM200 electron microscope.

Single-conidial isolates. Single-conidial cultures from isolate TN were analyzed for dsRNA. To isolate spores, a suspension made from a 4-day-old PDA culture was diluted to 10^3 spores per milliliter and 0.2 ml was spread on PDA plates. After 24 hr at 25 C, single germinating spores were transferred to fresh media.

Representative single-spore isolates differing in dsRNA content were tested for pathogenicity. The isolates were cultured in PDB medium on a shaker for 4 days at 25–26 C. Spore suspensions were adjusted to 10^6 spores per milliliter and 0.5 ml was injected into the stem of 3-yr-old rooted American elm (*Ulmus americana* L.) cuttings using the technique of Sterrett and Creager (32). Inoculations were made in mid-June 1979, and the trees were kept in a greenhouse at 24 C. At 6 wk after inoculation, disease was assessed as the percentage of the crown showing leaf symptoms (wilting, chlorosis, or drying). At 12 wk, the percentage of the main stem showing dieback (death of buds and bark) was recorded.

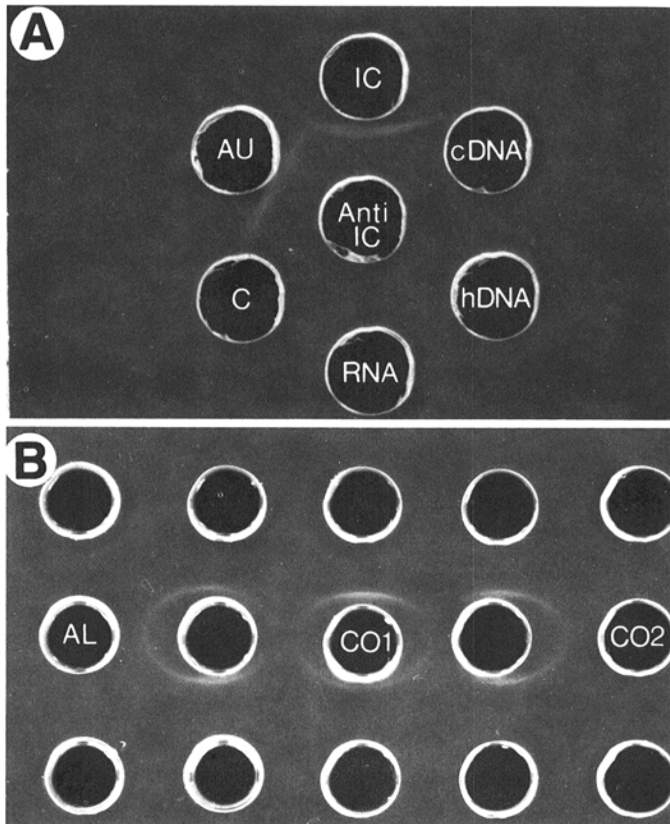

Fig. 1. Immunodiffusion tests using anti-dsRNA serum. **A,** Specificity of the anti-dsRNA serum (Anti IC) was determined by testing against poly I: poly C (IC), poly A: poly U (AU), poly C (C), yeast ssRNA (RNA), herring sperm DNA (hDNA), and calf thymus DNA (cDNA). Test antigens were used at concentrations from 4 to 6 A_{260nm} units per milliliter. **B,** Example of tests for dsRNA in *Ceratocystis ulmi* isolates. Anti-dsRNA serum was placed in the top and bottom rows of wells. Center wells contained antigens from *C. ulmi* isolates AL, CO1, and CO2. Alternate unmarked wells in center contained extracts from virus-infected *Penicillium chrysogenum.*

RESULTS

Serological detection of dsRNA. Specificity of the dsRNA antiserum for dsRNA was substantiated in tests involving various nucleic acids (Fig. 1A). Antiserum to poly I: poly C reacted with dsRNA (poly I: poly C and poly A: poly U), but not with ssRNA (poly C and yeast RNA) or DNA (herring sperm DNA and calf thymus DNA). Positive reactions indicating the presence of dsRNA in *C. ulmi* were observed for isolates of both the more aggressive and less aggressive groups (Fig. 1B, Table 1).

Electrophoretic analysis of dsRNA. Double-stranded RNA was detected in four of five less aggressive isolates and five of 10 more aggressive ones by polyacrylamide gel electrophoresis (PAGE) (Fig. 2). Results obtained by PAGE agreed with those of the serological tests with the exception of isolate CO2, in which dsRNA was detected by electrophoresis, but not by serology (Table 1). The same purification procedure was used for both detection methods, although preparations from different cultures were involved. For each isolate only one preparation was tested serologically and two

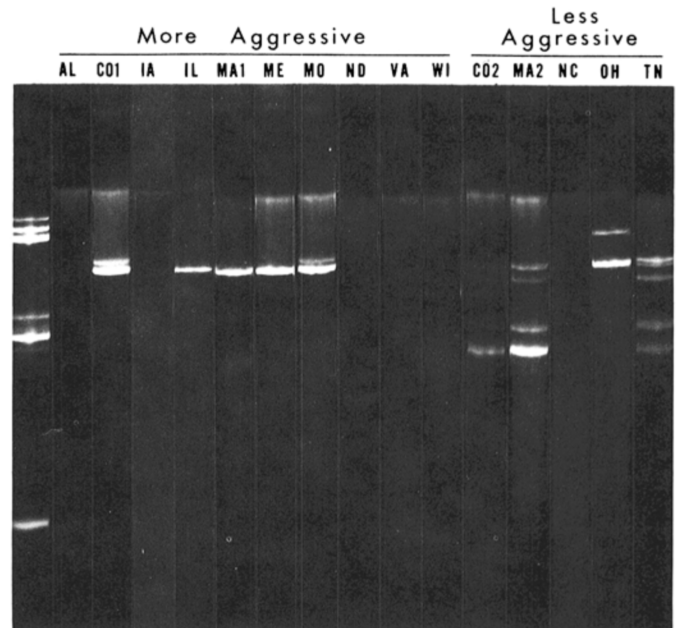

Fig. 2. Electrophoresis of dsRNA preparations from *Ceratocystis ulmi* isolates. The gel on the far left contains dsRNA from viruses in *Penicillium chrysogenum* (the upper three bands) and *P. stoloniferum* (the lower five bands). Other gels contain preparations from more aggressive (AL, CO1, IA, IL, MA-1, ME, MO, ND, VA, and WI) and less aggressive (CO2, MA2, NC, OH, and TN) isolates of *C. ulmi.* The fastest migrating bands from isolates CO2 and MA2, and the second fastest from isolate TN, were found later to contain doublets as shown in Fig. 3. The fastest band from TN, which appeared only in the first generation photograph for Fig. 2, is also quite visible in Fig. 3. Tests with nucleases showed that the uppermost fluorescent areas represent contaminating DNA. Gels were electrophoresed for 4.5 hr at 5 mA per gel.

or more preparations were examined using electrophoresis. The PAGE results showed that the size and number of dsRNA segments contained in isolates varied widely. In general, less aggressive isolates possessed a greater number of dsRNA segments than more aggressive ones. Three of the five less aggressive isolates had five to seven dsRNA segments, whereas none of the more aggressive ones had more than two dsRNA segments.

Nucleic acid bands stained with ethidium bromide were shown to be dsRNA by their resistance to DNase treatments, resistance to RNase at a high salt concentration (0.3 M NaCl), and susceptibility to RNase at a low salt concentration (0.01 M NaCl). The uppermost fluorescent areas, which consistently appeared in gels (Fig. 2), were eliminated only when treating with DNase. These areas apparently represent contaminating DNA.

To study the possible relatedness of dsRNA segments, *C. ulmi* isolates were initially placed in four groups according to dsRNA content. Group 1 contained isolates with one segment (IL, MA1 and ME), group 2 contained isolates with two segments closely banded (CO1, MO), group 3 contained isolates with two segments more widely separated (OH), and group 4 contained isolates with several segments that formed a similar banding pattern (CO2, MA2, and TN). Double-stranded RNA from one isolate in each group was coelectrophoresed with dsRNA from the other isolates in the same group; in all cases similar segments in the different isolates within a group comigrated and had the same molecular weight within experimental error. DsRNA preparations from representative isolates of different groups were then electrophoresed in the same gel. It was found that the single segment of group 1 comigrated with the faster segment of group 2 and with the slowest segment common to all isolates in group 4. The faster segment of group 3 comigrated with the slower segment of group 2, but moved slower than segments in group 4. To estimate molecular weights, preparations from representative isolates were coelectrophoresed with known molecular weight dsRNAs from *P. chrysogenum* virus (PcV) and *P. stoloniferum* virus (PsV). Molecular weight values used for the three segments of PcV-dsRNA were 2.18, 1.99, and 1.80×10^6 daltons; values used for the five segments of PsV-dsRNA were 1.11, 0.99, 0.94, 0.89, and 0.46×10^6 daltons (25). Approximate molecular weights of the unknowns (Table 2) were determined from a standard curve plotting electrophoretic mobility versus log of the molecular weight.

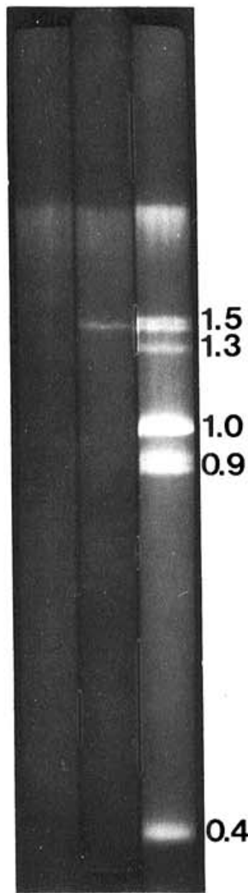

Fig. 3. Electrophoresis of dsRNA preparations from single-conidial isolates of *Ceratocystis ulmi* isolate TN. Gels are representative of three dsRNA segregant types when 12 single-spore isolates were examined. Approximate molecular weights ($\times 10^6$ daltons) are indicated on the right. Doublets are at 1.5 and 0.9×10^6 daltons. Gels were electrophoresed for 4.5 hr at 5 mA per gel.

TABLE 2. Approximate molecular weights of dsRNA segments detected in isolates of *Ceratocystis ulmi* by polyacrylamide gel electrophoresis

		Molecular weight[b]							No. of
Isolate	Pathogenicity[a]	0.4	0.9	1.0	1.3	1.5	1.6	2.0	segments
TN	L	+[c]	++[d]	+	+	++			7
CO2	L		++	+	+	+			5
MA2	L		++	+	+	+			5
OH	L						+	+	2
NC	L								0
CO1	M					+	+		2
MO	M					+	+		2
IL	M					+			1
MA1	M					+			1
ME	M					+			1
AL	M								0
IA	M								0
ND	M								0
VA	M								0
WI	M								0

[a] Abbreviations: M = more aggressive; L = less aggressive.
[b] Approximate molecular weight ($\times 10^6$ daltons).
[c] Indicates specific dsRNA segments present.
[d] Indicates doublet or two dsRNA segments of approximately the same molecular weight.

Attempts to detect VLPs. VLPs were detected in the standards, but not in isolates TN, MA2, or ND, regardless of the extraction or purification method used. Exhaustive electron microscopic examination of sucrose density gradient fractions for recognizable VLPs was negative. However, it is possible that VLPs exist in low titer and escaped detection or were not purified by the methods used.

Single-conidial isolates. Electrophoretic analysis of 12 single conidial isolates derived from isolate TN showed that seven of the 12 contained no dsRNA, two of the 12 contained one dsRNA segment (1.5×10^6), and only three of the 12 isolates contained seven dsRNA segments as did the parent isolate (Fig. 3). The dsRNA-segregant types were also found to be fairly distinguishable according to cultural characteristics. After several days on PDA at 25 C, all single-spore isolates having seven dsRNA segments produced isolated clumps of aerial mycelium near the edge of colonies; sometimes aerial growth was abundant near the colony margin, forming an uninterrupted ring. These cultures were like that of the parent TN isolate, except aerial mycelium of the parent was not observed as an uninterrupted ring. All isolates having only one dsRNA segment exhibited a striate growth pattern, would

TABLE 3. Pathogenicity of dsRNA-segregant types from *Ceratocystis ulmi* isolate TN[a]

Isolate[b]	No. dsRNA species	Crown symptoms after 6 wk (%)	Crown dieback after 12 wk (%)
TN-1	7	27 y[c]	16 y
TN-2	1	60 z	46 z
TN-3	0	62 z	49 z

[a] Twelve 3-yr-old rooted cuttings of *Ulmus americana* were inoculated in June 1979.
[b] Single-conidial isolates from isolate TN.
[c] Values in the same column followed by a different letter are significantly different according to Duncan's multiple range test ($P = 0.05$).

occasionally produce scattered eruptions of aerial mycelium, and a yellowing of the PDA medium was often noted. Cultures of isolates having no dsRNA produced aerial mycelium that was dispersed uniformly. Growth rate of the three segregant types was about the same. A striate growth pattern and an abundance of aerial mycelium are generally characteristics of more aggressive isolates (15,19). However, *C. ulmi* isolates used in the dsRNA analyses appeared to vary widely in cultural characteristics and were not distinquishable as more aggressive or less aggressive on this basis.

Results of inoculations involving one isolate of each segregant type are shown in Table 3. The severity of disease caused by isolate TN-1 (which has seven dsRNA segments) was significantly less than for isolates TN-2 and TN-3 (which have one dsRNA and no dsRNA, respectively). Because trees of susceptible age were in limited supply, only one isolate of each type was tested.

DISCUSSION

Detection of dsRNA in both more aggressive and less aggressive isolates of *C. ulmi* indicates that the presence of dsRNA is not always related to lowered pathogenicity. However, the additional dsRNA segments unique to less aggressive isolates may contribute to their decreased pathogenicity. These additional dsRNA segments may not be the only cause of low pathogenicity, since the less aggressive isolate NC lacks detectable dsRNA. It is possible that isolate NC requires a different set of growth conditions for dsRNA to be at a detectable level, or it could have lost dsRNA while in culture.

Isolate TN had the greatest number of dsRNA segments and was the least aggressive isolate in inoculation experiments by Schreiber and Townsend (30). Also, the most aggressive isolates, ND and AL, were among the isolates having no dsRNA. Isolate ME, with one dsRNA segment, was classified as more aggressive in this study, but was actually intermediate in aggressiveness according to Schreiber and Townsend (30).

A tendency of isolate CO2 to sector may explain why dsRNA was detected in it by electrophoresis, but not by serology. When three sector variants were examined for dsRNA by electrophoresis, dsRNA was detected in two of the variants and no dsRNA was detected in the other variant. The cultures used in the serological test may have been dominated by a variant having no dsRNA.

Most mycoviruses contain segmented dsRNA genomes. However, it is not known whether the dsRNAs in *C. ulmi* are associated with virus particles. Although no VLPs were detected in two dsRNA-containing isolates (TN and MA2), it would be premature to exclude viruses as the dsRNA source.

Transmission of dsRNA from one *C. ulmi* isolate to another has not been demonstrated. In *E. parasitica* (11) and *R. solani* (9) dsRNA transmission has been shown to occur through hyphal anastomosis between compatible strains. Possibly the dsRNA detected in *C. ulmi* is also transmitted in this way.

The discovery that seven of 12 conidia isolated from the TN isolate were free of dsRNA parallels findings by Day et al (11) for hypovirulent strains of *E. parasitica*. Similarly, about 10% of colonies derived from single spores of some strains of virus-infected *P. chrysogenum* were virus-free (10). Two other single-conidial selections from TN in the same experiment possessed only one of the seven dsRNA segments found in the parent isolate. Similar partial transmission of dsRNA through conidia has also been observed in *E. parasitica* (11).

A correlation between colony phenotype and dsRNA content was noted for the 12 single-spore isolates from TN. This again is comparable to work with *E. parasitica* in which single spores from white hypovirulent strains give rise to white (dsRNA-containing) and pigmented (dsRNA-free) forms (11). It is not known whether genetic alterations other than dsRNA content were important in the phenotypic changes of isolate TN.

Of the single-conidial isolates of TN, one with seven dsRNA segments was less pathogenic than another with one or a third with no dsRNA. These limited results offer additional evidence that diminished pathogenicity in *C. ulmi*, at least for isolate TN, is caused by the presence of some of the dsRNA segments. The pathogenicity of the single-spore isolates having no dsRNA was not statistically different from the isolate with one dsRNA segment. The dsRNA segment of the latter isolate had the same approximate molecular weight (1.5×10^6 daltons) as the segment in some of the more aggressive isolates. This molecular weight was the most common among *C. ulmi* isolates examined and may represent a segment athogenicity.

In summary, it is suggested that dsRNA can influence pathogenicity in *C. ulmi*. It appears, however, that the various dsRNA segments differ in their contribution to diminished pathogenicity and that other unknown factors, in addition to dsRNA, influence pathogenicity as well. The dsRNA may be contained in virus particles, although VLPs were not detected.

LITERATURE CITED

1. Atanasoff, D. 1973. The effect of superparasites on the pathogenicity of their hosts. Phytopathol. Z. 78:182-186.
2. Bevan, E. A., and Mitchell, D. J. 1979. The killer system in yeast. Pages 161–199 in: P. A. Lemke, ed. Viruses and Plasmids in Fungi. Marcel Dekker, New York. 653 pp.
3. Bozarth, R. F., Wood, H. A., and Mandelbrot, A. 1971. The *Penicillium stoloniferum* virus complex: Two similar double-stranded RNA virus-like particles in a single cell. Virology 45:516-523.
4. Brasier, C. M. 1977. Inheritance of pathogenicity and cultural characters in *Ceratocystis ulmi:* Hybridization of protoperithecial and non-aggressive strains. Trans. Br. Mycol. Soc. 68:45-52.
5. Brasier, C. M., and Gibbs, J. N. 1975. Variation in *Ceratocystis ulmi:* Significance of the aggressive and nonaggressive strains. Pages 53–66 in: Proc. Int. Union For. Res. Organ. Conf., September 1973, Minneapolis-St. Paul, MN. 94 pp.
6. Brasier, C. M., and Gibbs, J. N. 1976. Inheritance of pathogenicity and cultural characters in *Ceratocystis ulmi:* Hybridization of aggressive and non-aggressive strains. Ann. Appl. Biol. 83:31-37.
7. Castanho, B., and Butler, E. E. 1978. Rhizoctonia decline: A degenerative disease of *Rhizoctonia solani*. Phytopathology 68: 1505-1510.
8. Castanho, B., and Butler, E. E. 1978. Rhizoctonia decline: Studies on hypovirulence and potential use in biological control. Phytopathology 68:1511-1514.
9. Castanho, B., Butler, E. E., and Shepherd, R. J. 1978. The association of double-stranded RNA with Rhizoctonia decline. Phytopathology 68:1515-1519.
10. Crosse, R., and Mason, P. J. 1974. Virus-like particles in *Penicillium chrysogenum*. Trans. Br. Mycol. Soc. 62:603-610.
11. Day, P. R., Dodds, J. A., Elliston, J. E., Jaynes, R. A., and Anagnostakis, S. L. 1977. Double-stranded RNA in *Endothia parasitica*. Phytopathology 67:1393-1396.
12. Dodds, J. A. 1980. Double-stranded RNA and virus-like particles in *Endothia parasitica*. Association of type I viral-like dsRNA with club-shaped particles in hypovirulent strains of *Endothia parasitica*. Virology 107:1-12.
13. Francki, R. I. B., and Jackson, A. O. 1972. Immunochemical detection of double-stranded ribonucleic acid in leaves of sugarcane infected with Fiji disease virus. Virology 48:275-277.
14. Franklin, R. M. 1966. Purification and properties of the replicative intermediate of the RNA Bacteriophage R17. Proc. Nat. Acad. Sci., USA 55:1504-1511.
15. Gibbs, J. N., and Brasier, C. M. 1973. Correlation between cultural characters and pathogenicity in *Ceratocystis ulmi* from Britain, Europe and America. Nature 241:381-383.
16. Grente, J. 1965. Les formes hypovirulentes d'*Endothia parasitica* et les espoirs de lutte contre le chancre du chataignier. C. R. Seances Acad. Agric. Fr. 51:1033-1037.
17. Grente, J., and Sauret, S. 1969. L'hypovirulence exclusive, phénomène original enpathologie végétale. C. R. Acad. Sci. Paris 268:2347-2350.
18. Grente, J., and Sauret, S. 1969. L'hypovirulence exclusive est-elle controllée par des dêterminants cytoplasmiques. C. R. Acad. Sci. Paris 268:3173-3176.
19. Hindal, D. F., Harner, E. J., and MacDonald, W. L. 1979. Further studies on the relationship between cultural characteristics and pathogenicity in *Ceratocystis ulmi*. Phytopathology 69:108-111.
20. Hindal, D. F., and MacDonald, W. L. 1978. Production of synnemata on defined agar media and its relation to pathogenicity of *Ceratocystis ulmi*. Phytopathology 68:571-575.
21. Hollings, M. 1978. Mycoviruses: Viruses that infect fungi. Adv. Virus Res. 22:3-53.

22. Hollings, M., Stone, O. M., Barton, R. J., and Atkey, P. T. 1974. Dutch elm disease. Page 124 in: Reports of the Glasshouse Crops Research Institute for 1973.
23. Hollings, M., Stone, O. M., Barton, R. J., and Thomas, P. 1975. Viruses in plant pathogenic fungi. Page 123 in: Report of the Glasshouse Crops Research Institute for 1974.
24. Koltin, Y., and Levine, R. 1979. Fungal viruses and killer factors—*Ustilago maydis* killer proteins. Pages 120–128 in: H. P. Molitoris, M. Hollings, and H. A. Wood, eds. Fungal Viruses. Springer-Verlag, New York. 194 pp.
25. Lemke, P. A. 1977. Fungal viruses in agriculture. Pages 159–175 in: Beltsville Symposium in Agricultural Research. I. Virology in Agriculture. Alanheld, Osmun and Co., Montclair, NJ. 293 pp.
26. Lemke, P. A. 1977. Double-stranded RNA viruses among filamentous fungi. Pages 515–520 in: D. Schlessinger, ed. Microbiology—1977. Am. Soc. Microbiol., Washington, DC. 593 pp.
27. Moffitt, E. M., and Lister, R. M. 1973. Detection of mycoviruses using antiserum specific for dsRNA. Virology 52:301-304.
28. Moffitt, E. M., and Lister, R. M. 1975. Application of serological screening test for detecting double-stranded RNA mycoviruses. Phytopathology 65:851-859.
29. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
30. Schreiber, L. R., and Townsend, A. M. 1976. Variability in aggressiveness, recovery, and cultural characteristics of isolates of *Ceratocystis ulmi*. Phytopathology 66:239-244.
31. Stark, W. M., Kleinschmidt, W. J., and Probst, G. W. 1963. Statolon production by submerged culture fermentation. U.S. Patent 3,108,047.
32. Sterrett, J. P., and Creager, R. A. 1977. A miniature pressure injector for deciduous woody seedlings and branches. Hortic. Sci. 12:156-158.
33. Van Alfen, N. K., Jaynes, R. A., Anagnostakis, S. L., and Day, P. R. 1975. Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica*. Science 189:890-891.
34. Van Zaayen, A. 1979. Mushroom viruses. Pages 239–324 in: P. A. Lemke, ed. Viruses and Plasmids in Fungi. Marcel Dekker, New York. 653 pp.
35. Wood, H. A., Bozarth, R. F. and Mislivec, P. B. 1971. Viruslike particles associated with an isolate of *Penicillium brevi-compactum*. Virology 44:592-598.