Proceedings of the Iowa Academy of Science

Volume 77 | Number

Article 5

1970

Toxin Production By Isolates of Ceratocystis ulmi

Marcia V. Angell
Iowa State University

Harold S. McNabb Jr.
Iowa State University

Toxin Production By Isolates of *Ceratocystis ulmi*[1]

MARCIA V. ANGELL AND HAROLD S. MCNABB, JR.[2]

Abstract. Cuttings of two elm species, *Ulmus pumila* and *U. hollandica* 'Belgica', were used to assay toxin production by nine isolates of *Ceratocystis ulmi* representing collections from Asia, Europe, and North America. After 17 days of fungus growth in liquid culture, filtrates were collected and bioassayed by visual symptom expression over an approximate 10-hour period. A variation in toxin production was evident. One of the isolates from the Netherlands produced a low toxin titer. The other isolate from the Netherlands and the one from Asia produced highly significant symptoms on the resistant Siberian elm shoots and an Iowa isolate's filtrate reaction was significant on this elm species.

Little critical research has been done on the variation of virulence among isolates of *Ceratocystis ulmi* (Buisman) C Moreau. The fact that the resistance to Dutch elm disease found in a number of the world species of *Ulmus* has not suddenly broken down gives us little confidence of a uniform pathogen population because these resistant species have not been widely grown. The literature on virulence of *C. ulmi* is conflicting. Only small differences in pathogenicity were found among strains in Holland (12) and between a brown isolate and a normal cream-colored isolate in the United States (11). Monoconidial isolates from eight sectors in cultures of the pathogen exhibited a wide range of pathogenicity (10). Of these monoconidial isolates, the black variant was highly pathogenic and the nonsporulating isolate was avirulent. An isolate obtained from an artificially inoculated elm of the resistant selection, *Ulmus carpinifolia* Gled. 'Christine Buisman', proved to be virulent towards this resistant elm (7). This same Buisman isolate produced a larger proportion of non-volatile acids from the carbon source in liquid media than four other isolates (3). Recent studies in Holland also showed a wide range in virulence among monoconidial isolates of *C. ulmi* (9). Single ascospore cultures of artificially developed hybrids were more pathogenic than their parents (9). Physiological specialization possibly occurred too because individual isolates showed high degree of virulence on one elm clone and low degree of virulence on another elm clone (9). Single-ascospore isolates from progeny between type A strain from Massachusetts and type B from Holland varied widely in pathogenicity towards greenhouse grown elms (4). Similar individuals from a cross between two isolates from

[1] Journal Paper No. J-6743 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1706. Supported in part by an Emergency Grant from the Executive Council of Iowa.

[2] Granduate Teaching Assistant and Professor respectively, Department of Botany and Plant Pathology, Iowa State University of Science and Technoloygy, Ames.

Holland produced a fairly uniform level of symptoms in nursery grown trees (4).

Degree of symptom expression on growing trees in Dutch elm disease is related to a number of factors besides pathogen virulence: available moisture, air temperature, available nutrients, to mention a few. Therefore, other means of indicating degrees of virulence among a group of isolates would be helpful. Measuring toxin production by *C. ulmi* isolates is an example (6). Culture filtrates of a dark isolate from Sweden were more toxic to tomato plants than filtrates from normal appearing strains growing in the same area (5). Among eight isolates with different appearance in culture from United States, toxin titer was not correlated with variations in virulence (1). Our preliminary study presented here is another attempt to determine possible variability in virulence among isolates of *C. ulmi* by assuming a direct relationship between toxin titer and pathogenicity.

Materials and Methods

Nine isolates of *C. ulmi* representing a wide geographical distribution (Table 1) and various morphological differences were obtained from previously prepared stock cultures stored on solid elm extract medium (2) at 5°C. Mass transfers were made on slants of solid elm-extract medium to increase the amount of fungal material. After 2-weeks of growth at 25°C, 15 ml of sterilized, distilled water were poured into each slant culture and the tubes whirled until the liquid was cloudy with spores. These spore suspensions were returned to the respective flasks. Spore counts were taken for each flask by

Table 1. Origin of isolates of *Ceratocystis ulmi* used for toxin production

Experiment no. for isolate	Original isolate no.	Origin
1	TX - 51	Lower elevations of Indian Himalayas, Asia from H. Heybroek
2	TX - 50A	Holland, Europe Type A from H. Heybroek
3	TX - 21B	Holland, Europe Type B from H. Heybroek
4	Car A	Massachusetts Type A from F. Holmes
5	Marsh B	Massachusetts Type B from F. Holmes
6	229	Clarinda, Iowa Type B from M. Townsell
7	273	Clear Lake, Iowa Type A from M. Townsell
8	229 x 273-1	Hybrid from M. Townsell
9	229 x 273-2	Hybrid from M. Townsell

means of a Levy-Hausser Counting Chamber. Dilutions were made with small amounts of sterile, distilled water until each ml of all the suspensions contained approximately equal amounts of spores.

The liquid medium used for growth and toxin production by the 9 isolates of *C. ulmi* was Tchernoff's modification of the medium developed by Zentmyer (Table 2) (9). This medium was filtered and dispensed in 40 ml amounts into each of forty 125-ml flasks. The 40 flasks containing the medium were sterilized and stored at 4°C until needed.

Table 2. Growth medium, a modification of Zentmyer's by Tchernoff, for *Ceratocystis ulmi* used for toxin production

Chemical[1]	Amount per liter
Dextrose	20 g
L-asparagine	2 g
KH_2PO_4	1.5 g
$MgSO_4 \cdot 7H_2O$	1 g
$ZnSO_4 \cdot 7H_2O$	20 mg
$FeCl_2 \cdot 6H_2O$	10 mg
Pyridoxine hydrochloride	1 mg
Thiamine hydrochloride	1 mg

[1]all dissolved in 1 liter distilled waer

Into each flask containing liquid medium, 1 ml of spore suspension was placed aseptically. For each isolate, four flasks were seeded. The remaining four flasks were used as a check. The prepared flasks were placed on a shaker rotating at a slow speed at 25°C. Period of growth was for 17 days; cloudiness appearing in all flasks, except checks, after 2 days. None of the flasks showed contamination.

Vials holding 2 ml were used in assaying the culture filtrates. These filtrates were prepared by centrifuging together the material from the 4 flasks for each isolate or check, in a Sorvall Centrifuge for 20 minutes, at 4°C at 3000 g. Upon completion of centrifugation, the supernatant was filtered with the Seitz Bacteriological filter to remove any remaining spores or mycelia. The final filtrates were placed in the vials for assay. The remainder was flash frozen and stored at -24°C for future reference.

Elm shoot material was used to bioassay the culture filtrates for toxin activity. Two methods were used to produce shoots. For *U. hollandica* Mill. 'Belgica', shoots from root callus cuttings were used (8). Of the 352 root sections used, only 30.7% produced usable shoots in 52 days, although 47% callused (9). The Siberian elm (*U. pumila* L.) trees were obtained from a wholesale nursery in the dormant state and planted in pots in the greenhouse. Shoot

growth began within six weeks. The Belgica elm shoots were genetically homogeneous. Six Siberian elm trees were used, a tree producing the shoots for each of the six used in the test.

The desired shoots were cut, cut again under distilled water to insure preservation of water uptake, and the cut end inserted in the vials containing test filtrate. Each shoot was 4 to 6 cm long with two leaves and a leafy tip. For Belgica elm shoots, four replications were used. During the next 20 hours under artificial light, observations on shoot condition were made periodically and recorded (Table 3).

Table 3. Wilt index scale used to quantify symptom expression on treated cuttings

Index number	Symptom expression
1	No symptoms
2	Leaves spotty or chlorotic around edges, beginning to curl
3	Leaves wilted
4	All leaves seeverely wilted and dry
5	Leaves fallen

Results and Discussion

Although observations were made for a longer period, high transpiration rates and evaporation from the filtrate surface caused problems after about 10 hours. Therefore, the data collected at 8½ hours for Belgica elm and 9½ hours for Siberian elm are used for comparison (Table 4). The means of the index ratings for the filtrate of each isolate were tested for statistical significance within each species of cutting by means of Dunnett's t test. Siberian elm is considered resistant to Dutch elm disease. The data collected on symptom expression resulting from the toxin produced by the nine isolates indicate this resistance. The Dutch isolate type B and the Asiatic isolate produced symptoms that differed significantly from those of the check at the 1% level, and the Clear Lake type A, at the 5% level, on the resistant Siberian shoots. This can be explained by either a higher toxin titer in the filtrates from these isolates or a quality difference in toxin production. Different metabolites may be produced by these isolates that are able to cause symptoms in Siberian elm shoots.

The data clearly indicate the susceptibility of Belgica elm to Dutch elm disease. Several million elms of this selection have died from this disease in The Netherlands since 1920. The data also clearly indicate the low toxin titer of the Dutch isolate type A.

Our study supports to a degree the variation in toxin production by different isolates of *C. ulmi*. We also can suggest the possibility of difference in toxin quality from the data on the shoots of the

Table 4. Sums and means of wilt index readings for Siberian (after 9½ hr.) and Belgica (after 8 hr.) elm cuttings exposed to culture filtrates of *Ceratocystis ulmi* isolates

Isolate No. (Table 1)	Siberian elm cuttings' index rating[1] (Table 3) Sums	Means	Belgica elm cuttings' index rating[2] (Table 3) Sums	Means
1	14	2.33**	9	2.25**
2	9	1.50	5	1.25
3	17	2.83**	10	2.50**
4	10	1.66	7.5	1.88*
5	8.5	1.42	9	2.25**
6	9.5	1.58	10	2.50**
7	12	2.00*	10.5	2.63**
8	10	1.66	7.5	1.88*
9	10	1.66	8	2.00**
10[3]	6	1.00	4	1.00

** Level of significance=1%: Dunnett's t test
* Level of significance=5%; Dunnett's t test
[1] Sums and means of six replicates
[2] Sums and means of four replicates
[3] Sterilized culture medium without fungal growth (check)

resistant Siberian elm. Both these possibilities are of concern because of present efforts to produce and distribute new selections and hybrids of elms resistant to Dutch elm disease. Will this resistance be maintained if the population of *C. ulmi* has a wide variability in virulence? Much more study needs to be done on the natural population of *C. ulmi* and its pathogenic potential on present resistant elms.

Literature Cited

1. Frederick, L. & F. L. Howard. 1951. *Phytopathology* **41**: 12-13.
2. Hart, J. H. 1960. *Plant Disease Reprtr.* **44**: 806-807.
3. Higgins, D. J. 1959. Ph.D. Thesis, Ohio State University Library, Columbus, Ohio (Univ. Microfilms No. 60-748.)
4. Holmes, F. W. 1965. *Neth. J. Plant Path.* **71**: 97-112.
5. Mathiesen-Kaarik, A. 1953. *Skogen* **40**: 2-3, 11.
6. Salemink, C. A., H. Rebel, L. C. P. Kerling, & V. Tchernoff. 1965. *Science* **149**: 202-203.
7. Swingle, R. U. 1950. *Proc. Nat. Shade Conf.* **26**: 38-42.
8. Tchernoff, V. 1963. *Acta Botanica Neerlandica* **12**: 40-50.
9. Tchernoff, V. 1965. *Acta Botanica Neerlandica* **14**: 409-452.
10. Tyler, L. J. & K. G. Parker. 1945. *Phytopathology* **35**: 257-261.
11. Walter, J. M. & C. May. 1937. *Phytopathology* **27**: 142-143.
12. Went, Johanna C. 1938. *Phytopath. Z.* **11**: 181-201.