



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Naturally occurring non cerato-ulmin producing mutants of Ophiostoma novo-ulmi are pathogenic but lack aerial mycelium

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Naturally occurring non cerato-ulmin producing mutants of Ophiostoma novo-ulmi are pathogenic but lack aerial mycelium / C. BRASIER; S. KIRK; S. TEGLI. - In: MYCOLOGICAL RESEARCH. - ISSN 0953-7562. - STAMPA. - 99:(1995), pp. 436-440. [10.1016/S0953-7562(09)80641-2]

Availability:

The webpage <https://hdl.handle.net/2158/310845> of the repository was last updated on

Published version:

DOI: 10.1016/S0953-7562(09)80641-2

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Naturally occurring non cerato-ulmin producing mutants of *Ophiostoma novo-ulmi* are pathogenic but lack aerial mycelium

C. M. BRASIER¹, SUSAN A. KIRK¹ AND STEFANIA Tegli²

¹ Forest Research Station, Alice Holt Lodge, Farnham, Surrey GU10 4LH, U.K.

² Istituto di Patologia e Zoologica Agraria e Forestale, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144, Firenze, Italy

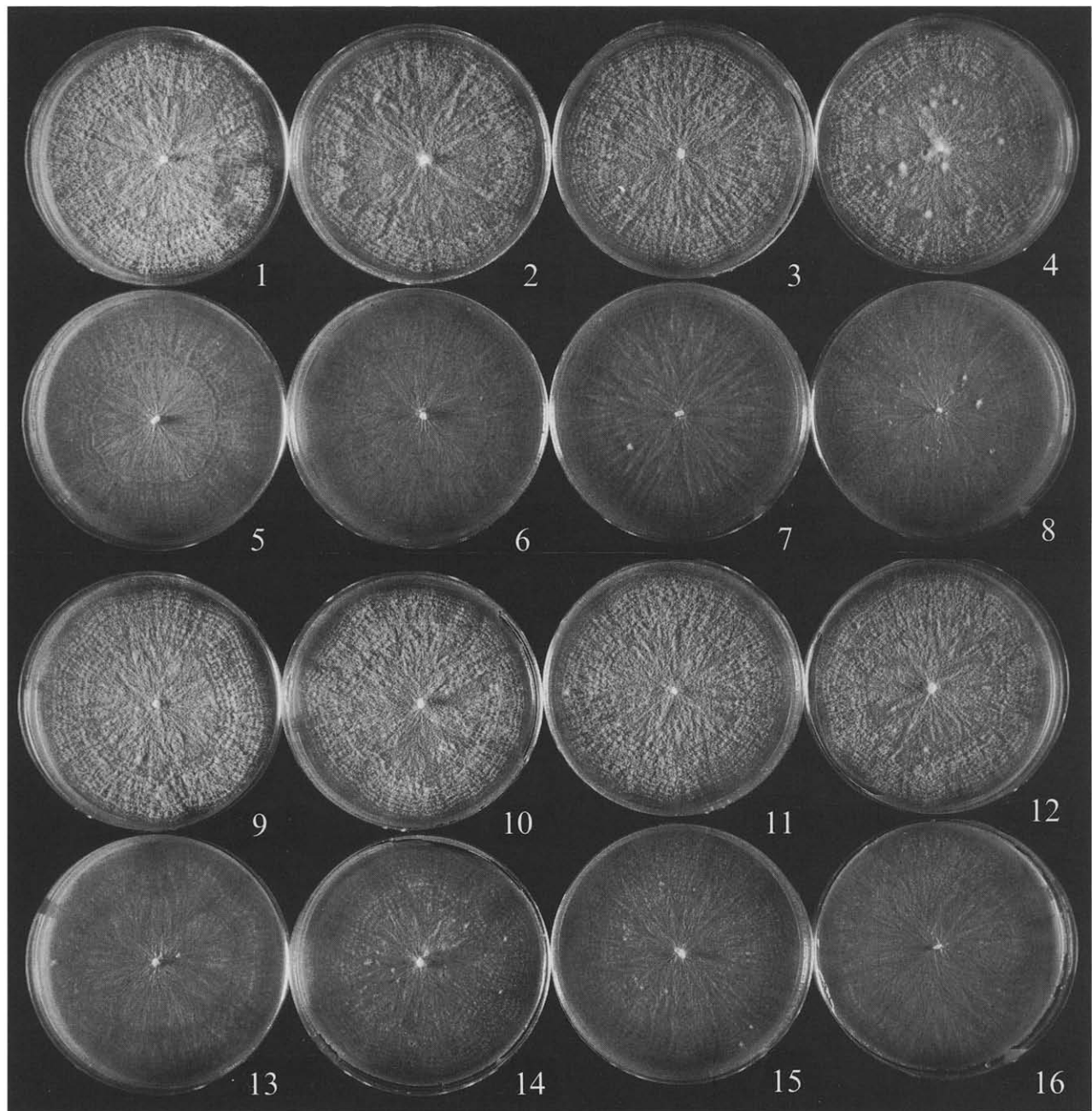
Cerato-ulmin is a protein implicated as a major toxin in the development of Dutch elm disease symptoms in elms infected with *Ophiostoma novo-ulmi*. *O. novo-ulmi* isolates typically produce fibrous-striate aerial mycelium on malt extract agar and secrete high levels of cerato-ulmin in liquid medium. However, two different genotypes of *O. novo-ulmi* (NAN race) from a population sample in Portugal, isolates MAFf8 and PG470, exhibited unusual flat-waxy 'non-aerial mycelial' colony types and were found to produce no detectable cerato-ulmin. The two isolates otherwise behaved as normal *O. novo-ulmi* isolates, including being highly pathogenic to *Ulmus procera*. When MAFf8 and PG470 were crossed with wild-type NAN *O. novo-ulmi* isolates, non-aerial mycelial colony phenotype and non cerato-ulmin production was shown in each case to be controlled by a single pleiotropic mutation, termed *cu*⁻. A further cross showed the *cu*⁻ locus was probably allelic in the two isolates. The influence of the *cu*⁻ locus on colony phenotypes in *O. novo-ulmi* supports the view that cerato-ulmin is a fungal hydrophobin. The normal pathogenic ability of the two *cu*⁻ isolates raises questions about the proposed role of cerato-ulmin as a wilt toxin.

Cerato-ulmin is a low molecular weight protein of c. 8000 daltons (Yaguchi *et al.*, 1992; Scala *et al.*, 1994) produced by the Dutch elm disease pathogens *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier. The molecule has unusual physical properties. Its individual 'units' assemble into rods and fibrils at air-water interfaces (Takai, 1974), it has a large proportion of hydrophobic residues (Stevenson, Slater & Takai, 1979), and from its apparent combination of hydrophobic and hydrophilic properties, it is considered a probable surfactant (Russo *et al.*, 1981). The protein is produced in considerable quantity in liquid cultures by the highly aggressive *O. novo-ulmi*, and at generally much lower levels by the less aggressive *O. ulmi* (Takai, 1974, 1978; Barrett & Skidmore, 1975; Brasier *et al.*, 1990; Tegli *et al.*, 1994). It also induces characteristic wilting and internal disease symptoms in elm cuttings, can be detected immunologically *in vivo* in infected elm shoots, and shows some host specificity to elm (Takai, 1974; Takai & Richards, 1978; Russo *et al.*, 1981, 1982; Richards & Takai, 1984; Okamoto *et al.*, 1986; Richards, 1993). It has therefore been proposed to be the major extracellular toxin involved in the development of vascular wilt symptoms in Dutch elm disease (Takai, 1974; Takai & Richards, 1978; Richards, 1993).

Cerato-ulmin also occurs on the hyphal surfaces, spore surfaces and in the synnemetal head fluid of *O. novo-ulmi* (Takai, 1980; Takai *et al.*, 1980; Svircev, Jeng & Hubbes, 1988). Moreover, Stringer & Timberlake (1993) have noted that the hydrophobicity plot of cerato-ulmin produced by Bolyard & Sticklen (1992), based on the amino acid sequence derived by Stevenson *et al.* (1979) and Yaguchi *et al.* (1992),

possesses the structural features of a hydrophobin. These are a recently discovered class of molecules considered to confer hydrophobicity to hyphal and spore surfaces, and implicated in spore dispersal and in pathogen adhesion processes (Beever & Dempsey, 1978; Doyle & Rosenberg, 1990; Stringer *et al.*, 1991). By using mutants Wessels *et al.* (1991a, b; see also Wosten, DeVries & Wessels, 1993; Wosten *et al.*, 1994) have demonstrated that in *Schizophyllum commune* hydrophobins are also involved in cell wall polymerization, and in the development of more differentiated non-assimilative hyphae from more juvenile assimilative hyphae. A general implication is that hydrophobins are a universal structural feature of fungal mycelia with important developmental functions (Wessels, 1992). This raises the issue of the comparative role of cerato-ulmin as a hydrophobin versus its role as a putative elm toxin.

Two fresh wild isolates of *O. novo-ulmi* from a population sample of several hundred NAN race isolates collected in Portugal during 1985 and 1986 (Brasier, 1988) were found to have an atypical colony phenotype for the species in Oxoid malt extract agar (MEA; see Brasier, 1981). Isolate PG470 was obtained from a fresh xylem infection in an elm at Mafra, and isolate MAFf8 from diseased elm bark of another tree near the same location. Both isolates exhibited a flat, waxy colony type lacking any significant development of aerial mycelium (Figs 5, 13) in contrast to the usual fibrous striate petaloid appearance of *O. novo-ulmi* NAN colonies (Figs 1, 9). MAFf8 was of sexual compatibility type A, and PG470 of type B, and the two isolates were each of a different vegetative incompatibility type (see Brasier, 1988). Hence they were of



Figs 1–16. Colony characteristics of parents and progenies in crosses between wild-type and 'non-aerial mycelial' NAN *O. novo-ulmi* isolates. Crosses: H351 × PG470 (Figs 1–8) and PG402 × MAff8 (Figs 9–16). **Fig. 1**, wild-type parent H351. **Figs 2–4**, wild-type F_1 s. **Fig. 5**, non-aerial mycelial parent PG470. **Figs 6–8**, non-aerial mycelial F_1 s. **Fig. 9**, wild-type parent PG402. **Figs 10–12**, wild-type F_1 s. **Fig. 13**, non-aerial mycelial parent MAff8. **Figs 14–16**, non-aerial mycelial F_1 s. The isolates were grown on Oxoid malt extract agar at 20 °C as described in Brasier (1981).

different background genotype, but of possibly related genetic origin via a sexual recombination event at the Mafra site.

It was initially suspected from their unusual colony phenotypes that MAff8 and PG470 might be products of rare introgression between *O. ulmi* and *O. novo-ulmi* as a result of the intermingling of the two species at current epidemic fronts (Brasier & Kirk, 1994). To examine this possibility, they were biologically characterized in terms of their temperature-growth relationships, sexual fertility barrier against *O. ulmi* both as donor (♂) and as a recipient (♀), vascular wilt ability in elm xylem, and pathogenicity to elm bark using the

methods described in Kile & Brasier (1990); and for their extracellular cerato-ulmin production index (CPI) in *in vitro* liquid cultures using the method of Takai & Richards (1978). Their pathogenicity to the susceptible *Ulmus procera* and to the more resistant Commelin elm (Table 1) fell within the normal range for *O. novo-ulmi* (see Brasier, 1986), and they conformed to *O. novo-ulmi* in all the other properties tested with the exception of their CPI. This was found to be close to zero in contrast to the normal high CPI readings of NAN *O. novo-ulmi* isolates. The two isolates were also shown to have typical NAN *O. novo-ulmi* nuclear DNA patterns following

Table 1. Pathogenicity of non-aerial mycelial and wild-type NAN *O. novo-ulmi* isolates

Isolates	Pathogenicity (mean % defoliation)* at 12 wk		Mean lesion area (cm ²) in <i>U. procera</i> bark at 10 wk
	2m <i>Ulmus procera</i>	3m <i>U.</i> × <i>Commelin</i>	
Non-aerial mycelial NAN isolates			
MAFf8	72.5	36.0	22.1 ± 11.6
PG470	80.0	41.1	14.1 ± 12.3
NAN wild-type controls**			
H351	nt	50.4	nt
PG402	66.8	71.9	15.6 ± 10.3‡
PG426	89.1	nt	13.9 ± 11.6
PG465	72.2	26.1	nt
<i>O. ulmi</i> control			
PG401	19.5	0	7.6 ± 6.1

*, Results of inoculation experiments conducted from mid-June to mid-September 1987.

**, Control isolates PG402, PG426, PG465 and PG401 are from Portugal (see Brasier, 1988; Kile & Brasier, 1990) and H351 from Belgium (see Brasier, 1986).

nt, Not tested.

‡, Lesion size of PG402 was assessed at 13 wk in a separate experiment.

*, Results of inoculation experiments conducted from mid-June to mid-September 1987.

**, Control isolates PG402, PG426, PG465 and PG401 are from Portugal (see Brasier, 1988; Kile & Brasier, 1990) and H351 from Belgium (see Brasier, 1986).

nt, Not tested.

‡, Lesion size of PG402 was assessed at 13 wk in a separate experiment.

random amplification of their DNA via the polymerase chain reaction using eleven random oligonucleotide probes which distinguish *O. ulmi* from *O. novo-ulmi* (N. Pipe, K. W. Buck and C. M. Brasier, unpublished observations).

The outstanding properties of PG470 and MAFf8 therefore were an absence of detectable extracellular cerato-ulmin production and a 'non-aerial mycelial' colony type. Their cerato-ulmin production was therefore retested using Scala *et al.*'s (1994) refinement of the turbidometric test of Takai & Richards (1978), in which the cerato-ulmin production index (CPI) of an isolates culture filtrate is calculated from a series of aqueous dilutions (1:1, 1:2, 1:3 to 1:n). Each dilution is first shaken to induce turbidity of the cerato-ulmin. Its optical density at 400 nm is then compared to that of a still sample at the same dilution, and plotted against the decimal logarithm of the dilution factor. It was confirmed that the mean CPI's of MAFf8 and PG470 lay close to zero (at 1.9 and 2.0 in this test) compared with the high CPI's of control NAN *O. novo-ulmi* isolates PG402 and ES376 (at 1080.2 and 1012.7 respectively). In the same test control *O. ulmi* isolate H200 gave a typically low mean CPI reading for *O. ulmi* of 8.6.

To investigate the genetic control of their unusual colony phenotype and non cerato-ulmin production, each isolate was crossed with an *O. novo-ulmi* isolate exhibiting a normal wild-type colony pattern and high level of cerato-ulmin production; PG470 with A-type isolate H351 (from Belgium; see Brasier, 1985) and MAFf8 with B-type isolate PG402 (from Portugal, see Kile & Brasier, 1990). Crosses and isolation of random ascospores were carried out as described by Kile & Brasier (1990). From each cross 102 and 100 progeny respectively were examined. They segregated in a near 1:1 ratio for wild type: non-aerial mycelial colony types (52:50 and 53:47 respectively), indicating the involvement of a single locus in each case. The two phenotypes also segregated independently of sexual compatibility type. Otherwise the progeny showed typical growth rate variation and in the case of the wild-type progeny typical colony patterns for F₁ progeny samples in crosses between NAN isolates (Figs 1–16), i.e. both isolates

'bred true' to *O. novo-ulmi*. Ten wild-type and 10 non-aerial mycelial progeny of each cross were then selected at random and tested for cerato-ulmin production using the method of Scala *et al.* (1994). All 20 non-aerial mycelial progeny isolates produced no detectable cerato-ulmin, whereas the 20 wild types produced high levels of cerato-ulmin comparable to that of PG402 and H351 (Table 2). This indicated that in each case a single locus was probably responsible both for the non-aerial mycelial phenotype and for the absence of cerato-ulmin production. To test for possible allelism of the loci in MAFf8 and PG470, these two isolates were crossed and 300 ascospore progeny characterized. All 300 F₁s exhibited the non-aerial mycelial phenotype. The loci involved are therefore most probably allelic. The locus has been designated the *cu*⁻ (non cerato-ulmin producing) locus, and its wild-type equivalent the *cu*⁺ locus.

This evidence for pleiotropic control of the non-production of a hydrophobin-like molecule and an absence of aerial mycelium parallels the more detailed observations made by Wessels *et al.* (1991a, b) with *Sc* mutants in *Schizophyllum commune*, and supports the view of Stringer & Timberlake (1993) that cerato-ulmin is a hydrophobin. It suggests that in *O. novo-ulmi*, cerato-ulmin may have a similar function in hyphal development and in spore protection to that proposed for hydrophobins in other fungi (Beever & Dempsey, 1978; Stringer *et al.*, 1991; Wessels, 1992). With the exception of the *cu*⁻ locus and its effects, MAFf8 and PG470 otherwise behave as normal *O. novo-ulmi* isolates. The identification of the *cu*⁻ locus therefore offers the opportunity to examine the effects of a hydrophobin on *O. novo-ulmi* development, as has already been done in *S. commune* and *Aspergillus* (e.g. Stringer *et al.*, 1991; Wessels, 1992; Wosten *et al.*, 1993, 1994). Of obvious interest would be to compare viability of conidia and ascospores and comparative development of fruiting structures in *cu*⁺ and *cu*⁻ isolates. Another approach could be to study the function of the *cu*⁻ allele by inserting it directly into *O. ulmi* by genetic transformation. It should be noted, however, that while MAFf8 *cu*⁻ and PG470 *cu*⁻ are most probably

Table 2. Cerato-ulmin production *in vitro* by non-aerial mycelial and wild-type NAN *O. novo-ulmi* isolates and their F₁ progenies

Parent and F ₁ isolates	Turbidity of shaken culture filtrate*	Cerato-ulmin production index (CPI)†		
		No. of isolates tested	Mean CPI	Range
Cross H351 × PG470				
Wild-type parent H351	High	1	230	117–318
Non-aerial mycelial parent PG470	Zero	1	0	
10 wild-type F ₁ s	All high	5	206	
10 non-aerial mycelial F ₁ s	All zero	10	0	
Cross PG402 × MAFf8				
Wild-type parent PG402	High	1	324	105–387
Non-aerial mycelial parent MAFf8	Zero	1	0	
10 wild-type F ₁ s	All high	5	240	
10 non-aerial mycelial F ₁ s	All zero	10	0	
<i>O. ulmi</i> controls				
R21	Near zero	1	5	
H200	Zero	5	0	

* , High turbidity, spectrophotometer reading (at 400 nm) > 2.5 at the 1:1 aqueous dilution. Zero turbidity, spectrophotometer reading (at 400 nm) < 0.004 at the 1:1 aqueous dilution.

† , Mean of two replicate dilution series.

* High turbidity, spectrophotometer reading (at 400 nm) > 2.5 at the 1:1 aqueous dilution. Zero turbidity, spectrophotometer reading (at 400 nm) < 0.004 at the 1:1 aqueous dilution.

†, Mean of two replicate dilution series.

naturally occurring mutants of *O. novo-ulmi*, there remains a possibility that their *cu*⁻ locus was derived from *O. ulmi* via rare introgression.

The present observations also raise interesting questions about the role of cerato-ulmin as a putative toxin during Dutch elm disease pathogenesis. MAFf8 *cu*⁻ and PG470 *cu*⁻ are as pathogenic to elm xylem or elm bark as most NAN *O. novo-ulmi* isolates (Table 1; Brasier, 1986). Barrett & Skidmore (1975) showed that white 'feltoid' cultural variants of NAN *O. novo-ulmi* (a spontaneous degenerate variant; cf. Brasier, 1982) also produced little or no cerato-ulmin *in vitro* yet remained highly pathogenic to elms. To account for cerato-ulmin's role as a toxin (Richards, 1993), it is possible that in the case of the *cu*⁻ and 'feltoid' isolates much higher levels of cerato-ulmin production are induced *in planta* than are produced *in vitro*, though this seems unlikely in view of the high constitutive CPI levels normally achieved by wild-type isolates in liquid cultures (cf. Table 2). It is also possible that the negligible *in vitro* CPI levels of the *cu*⁻ and feltoid isolates are sufficient for normal expression of pathogenicity *in vivo*. A further possibility is that, even in the absence of significant cerato-ulmin production, the remaining armoury of toxins and enzymes available to *O. novo-ulmi* is sufficient for pathogenic function. The above issue may be clarified if the levels of cerato-ulmin production by *cu*⁻ isolates *in planta* and *in vitro* can be compared by immunological methods. Critical to the success of such an approach, however, is the development of assays that are sufficiently sensitive and quantitative.

It is a characteristic of the less aggressive *Ophiostoma ulmi* that CPI levels in liquid cultures tend to be low or near zero (e.g. Brasier *et al.*, 1990). In this species, therefore, the molecule might function mainly as a cell surface hydrophobin. Unfortunately, interspecific crosses between *O. ulmi* and *O. novo-ulmi* show a rather complex pattern of inheritance for cerato-ulmin production (Brasier, 1987; Kile & Brasier, 1990) which could well reflect the fact that the protein is structurally different and differently regulated in the two species (Brasier

et al., 1990). Thus the typically higher *in vitro* CPI levels of *O. novo-ulmi* might be achieved through multiple copies of the relevant structural genes, through an alternative regulation system, or through a mechanism of enhanced cerato-ulmin excretion (cf. Sutherland & Brasier, 1994). Indeed the *cu*⁻ locus could conceivably be a locus affecting cerato-ulmin excretion ability rather than one directly affecting intracellular production levels of the protein.

REFERENCES

- Barrett, D. K. & Skidmore, A. M. (1975). Metabolite of *Ceratocystis ulmi* and its association with pathogenicity. *Transactions of the British Mycological Society* **65**, 469–475.
- Beever, R. E. & Dempsey, G. P. (1978). Function of rodlets on the surface of fungal spores. *Nature* **272**, 608–610.
- Bolyard, M. J. & Sticklen, M. D. (1992). Expression of a modified Dutch elm disease toxin in *Escherichia coli*. *Molecular Plant-Microbe Interactions* **5**, 520–524.
- Brasier, C. M. (1981). Laboratory investigation of *Ceratocystis ulmi*. In *Compendium of Elm Diseases* (ed. R. J. Stipes & R. J. Campana), pp. 76–79. American Phytopathological Society: St Paul, Minnesota.
- Brasier, C. M. (1982). Occurrence of three sub-groups within *Ceratocystis ulmi*. In *Proceedings of the Dutch Elm Disease Symposium and Workshop*, Winnipeg, Manitoba, 5–9 October 1981 (ed. E. S. Kondo, Y. Hiratsuka & W. B. C. Denyer), pp. 298–321. Manitoba Department of Natural Resources: Manitoba, Canada.
- Brasier, C. M. (1986). A comparison of pathogenicity and cultural characters in the EAN and NAN aggressive subgroups of *Ophiostoma ulmi*. *Transactions of the British Mycological Society* **87**, 1–13.
- Brasier, C. M. (1987). Some genetic aspects of necrotrophy with special reference to *Ophiostoma ulmi*. In *Genetics and Plant Pathogenesis* (ed. P. R. Day & G. J. Jellis), pp. 297–310. Blackwell Scientific Publications: Oxford.
- Brasier, C. M. (1988). Rapid changes in genetic structure of epidemic populations of *Ophiostoma ulmi*. *Nature* **332**, 538–541.
- Brasier, C. M. & Kirk, S. A. (1994). Rare hybrids may be a genetic bridge between *Ophiostoma ulmi* and *O. novo-ulmi*. *Report on Forest Research 1993*, p. 11. HMSO: London.
- Brasier, C. M., Takai, S., Nordin, J. H. & Richards, W. C. (1990). Differences in cerato-ulmin production between the EAN, NAN and non-aggressive sub-groups of *Ophiostoma ulmi*. *Plant Pathology* **39**, 231–236.

- Doyle, R. J. & Rosenberg, M. (1990). *Microbial Cell Surface Hydrophobicity*. American Society for Microbiology: Washington D.C.
- Kile, G. A. & Brasier, C. M. (1990). Inheritance and inter-relationship of fitness characters in progeny of an aggressive \times non-aggressive cross of *Ophiostoma ulmi*. *Mycological Research* **94**, 514–522.
- Okamoto, H., Otani, H. & Takai, S. (1986). Effect of cerato-ulmin (CU) on electrogenic pumps of elm cell membrane and ion loss from cells. *Phytopathology* **76**, 113 (Abstr.).
- Richards, W. C. (1993). Cerato-ulmin: a unique wilt toxin of instrumental significance in the development of Dutch elm disease. In *Dutch Elm Disease Research. Cellular and Molecular Approaches* (ed. M. B. Sticklen & J. L. Sberald), pp. 89–151. Springer-Verlag: New York.
- Richards, W. C. & Takai, S. (1984). Characterization of the toxicity of cerato-ulmin, the toxin of Dutch elm disease. *Canadian Journal of Plant Pathology* **6**, 291–298.
- Russo, P. S., Blum, F. D., Ipsen, J. D., Abul-Hajj, Y. J. & Miller, W. G. (1981). The solubility and surface activity of the *Ceratocystis ulmi* toxin cerato-ulmin. *Physiological Plant Pathology* **19**, 113–126.
- Russo, P. S., Blum, F. D., Ipsen, J. D., Abul-Hajj, Y. J. & Miller, W. G. (1982). The surface activity of the phytotoxin cerato-ulmin. *Canadian Journal of Botany* **60**, 1414–1422.
- Scala, A., Tegli, S., Comparini, C., Mittempergher, L., Scala, F. & Del Sorbo, G. (1994). Influence of fungal inoculum on cerato-ulmin production: purification of cerato-ulmin and detection in elm sucker cuttings. *Petria* **4**, 53–63.
- Stevenson, K. J., Slater, J. A. & Takai, S. (1979). Cerato-ulmin, a wilting toxin of Dutch elm disease fungus. *Phytochemistry* **18**, 235–238.
- Stringer, M. A., Dean, R. A., Sewell, T. C. & Timberlake, W. T. (1991). *Rodletless*, a new *Aspergillus* developmental mutant induced by directed gene inactivation. *Genes and Development* **5**, 1161–1171.
- Stringer, M. A. & Timberlake, W. E. (1993). Cerato-ulmin, a toxin involved in Dutch elm disease, is a fungal hydrophobin. *The Plant Cell* **5**, 145–146.
- Sutherland, M. L. & Brasier, C. M. (1994). D-infection affects cerato-ulmin toxin production by the Dutch elm disease fungus. *Report on Forest Research 1993*, pp. 11–12. HMSO: London.
- Svircev, A. M., Jeng, R. S. & Hubbes, M. (1988). Detection of cerato-ulmin on aggressive isolates of *Ophiostoma ulmi* by immunochemistry and scanning electron microscopy. *Phytopathology* **78**, 322–327.
- Takai, S. (1974). Pathogenicity and cerato-ulmin production in *Ceratocystis ulmi*. *Nature* **252**, 124–126.
- Takai, S. (1978). Cerato-ulmin, a wilting toxin of *Ceratocystis ulmi*: cultural factors affecting cerato-ulmin production by the fungus. *Phytopathologische Zeitschrift* **91**, 147–158.
- Takai, S. (1980). Relationship of the production of the toxin cerato-ulmin to synnemata formation, pathogenicity, mycelial habit and growth of *Ceratocystis ulmi* isolates. *Canadian Journal of Botany* **58**, 658–662.
- Takai, S., Hiratsuka, Y., Krywienczyk, J., Richards, W. C. & Davies, Y. P. (1980). Evidence for the presence of the toxin cerato-ulmin in the synnema head fluid of *Ceratocystis ulmi*. *Canadian Journal of Botany* **58**, 669–675.
- Takai, S. & Richards, W. C. (1978). Cerato-ulmin, a wilting toxin of *Ceratocystis ulmi*: isolation and some properties of cerato-ulmin from the culture of *C. ulmi*. *Phytopathologische Zeitschrift* **91**, 129–146.
- Tegli, S., Comparini, C., Giannetti, C. & Scala, A. (1994). Effect of temperature on growth and cerato-ulmin production of *Ophiostoma novo-ulmi* and *O. ulmi*. *Mycological Research* **98**, 408–412.
- Wessels, J. G. H. (1992). Gene expression during fruiting in *Schizophyllum commune*. *Mycological Research* **96**, 609–620.
- Wessels, J. G. H., de Vries, O. M. H., Ásgeirsdóttir, S. A. & Springer, J. (1991a). The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene. *Journal of General Microbiology* **137**, 2439–2445.
- Wessels, J. G. H., de Vries, O. M. H., Ásgeirsdóttir, S. A. & Schuren, F. H. J. (1991b). Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum*. *The Plant Cell* **3**, 793–799.
- Wosten, H. A. B., Ásgeirsdóttir, S. A., Krook, J. H., Drenth, J. H. H. & Wessels, J. G. H. (1994). The fungal hydrophobin *Sc3p* self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. *European Journal of Cell Biology* **63**, 122–129.
- Wosten, H. A. B., De Vries, O. M. H. & Wessels, J. G. H. (1993). Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer. *Plant Cell* **5**, 1567–1574.
- Yaguchi, M., Pusztai, M., Roy, C., Surewicz, W. K., Carey, P. R., Stevenson, K. J., Richards, W. C. & Takai, S. (1992). Amino acid sequence and spectroscopic studies of Dutch elm disease toxin, cerato-ulmin. In *Dutch Elm Disease Research. Cellular and Molecular Approaches* (ed. M. B. Sticklen & J. L. Sberald), pp. 152–170. Springer-Verlag: New York.