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Ophiostoma novo-ulmi sp. nov., causative agent of current Dutch elm disease pandemics

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Abstract

The aggressive subgroup of the Dutch elm disease pathogen *Ophiostoma ulmi* (Buism.) Nannf. syn. *Ceratocystis ulmi* (Buism.) Moreau is named as a new species, *O. novo-ulmi*, and is thereby separated from the 'old' non-aggressive subgroup, which is retained as *O. ulmi*. *O. novo-ulmi* differs from *O. ulmi* in colony morphology, growth rate, optimum temperature for growth, perithecial neck length, pathogenicity to elm, bark colonising ability, cerato-ulmin protein production, synnemetal and protoperithecial production, mating type frequency, protein and isozyme polymorphisms, mitochondrial DNA and nuclear DNA polymorphisms, and mitochondrial DNA size. In addition, a strong unidirectional fertility barrier operates between the two species, while their hybrids show remarkable variation, poor fitness, and many are infertile. These aspects are summarised. New information on perithecial dimensions is presented. *O. ulmi* is redefined and a neotype designated. The status of the Eurasian and North American races of *O. novo-ulmi* is currently under investigation.

Abbreviations: EAN – Eurasian race; NAN – North American race

Introduction

Dutch elm disease is one of the most destructive plant diseases in the Northern Hemisphere. There have been two pandemics of the disease in this century. The first began in north west Europe in the early 1900s, quickly spread eastwards to central and southern Europe and westwards to Britain and North America. It also spread later to south west and central Asia. This pandemic declined in Europe from the 1940s onwards. The second pandemic was recorded initially in Britain in the early 1970s, though it probably began rather earlier, possibly originating from a centre in eastern Europe near Romania and also spreading from a second centre in mid-western North America around the 1940s–1950s. It continues to spread, and in consequence the majority of mature elms are likely to be killed across much of North America, Europe, and central and south west Asia [13, 19, 22, 36].

The pathogen responsible for the first pandemic was isolated and characterised in the Netherlands by Bea Schwarz in 1922 and named

Table 1. Differences betweeen the aggressive and non-aggressive subgroups

	Non-aggressive *O. ulmi*	Aggressive (EAN + NAN races) *O. novo-ulmi*	References
Morphological			
Perithecial neck length (elm twigs)	280–420 μm	230–640 μm	See Table 2
Perithecial neck length/base width ratio	2.4–3.5	1.5–6.2	See Table 2
Colony morphology at 20 °C	——Distinctive——		9, 11, 24, 25, 26, 37, 39, 53, 54
Physiological			
Radial growth rate (mm/day^{-1}) of wild-type isolates at 20 °C	2.0–3.1	3.1–4.8*	9, 11, 15, 24, 25, 27, 37, 39 42, 51, 53, 54
Growth optimum °C	~(25–)27.5–30 °C	~20–22 °C	27, 48
Growth maximum °C	35 °C	32–33 °C	27
Pathogenicity (% defoliation)			6, 10, 11, 15, 26, 37, 38, 48
on clonal *Ulmus* procera	10–35% (–40%)	60–100%	
	Recovery normal	Recovery scarce	
on *U.* × 'Commelin'	0%	40–90%	
Bark colonising ability	Weak	Strong	48, 50, 67
Cell wall degrading ability	Poor	Good	56, 61
Cerato-ulmin production	Nil–very low	Moderate-high	1, 29, 55, 57, 62, 63
Synnematal production on elm sapwood	None (–few)	Occasional-frequent	47, 48, 58, 63
Ascogonial/protoperithecial production (MEA)	Nil	Occasional (B-types) To frequent (A-types)	11, 14, 18
Mating type frequency in nature	A and B-types equal (Eurasia) or A-type predominant (North America)	B-type predominant	14, 21, 23, 26, 50
Fertility reaction as ♀	Accepts aggressive	Strongly rejects non-aggressive	6, 7, 14, 48
Vegetative incompatibility reactions	n or w – reactions often indistinct	distinct n and w – reactions	14, 50
Molecular			
Monoclonal antibodies	——Some specific——		33
Protein and isozyme polymorphisms	——Distinctive——		5, 44, 47
Mitochondrial DNA size	74–88 kb	48–71 kb	2, 3
Mitochondrial DNA polymorphisms	——Very distinctive——		2, 4, 46
Nuclear DNA polymorphisms	——Very distinctive——		2, 4

Ranges given are common ranges only and frequencies given are 'norms'. For extremes see references and Table 2.

* *N.B.* The '*up-mut*' colony form of the EAN aggressive may grow more slowly [15]. MEA = 'Oxoid' malt extract agar [9]. ESA = elm sapwood agar [9].

by her as *Graphium ulmi* from its synnemetal state [59]. In 1932 the sexual stage was identified by Christina Buisman and described by her as *Ceratostomella ulmi* [31]. It was transferred to *Ophiostoma* by Nannfeldt [49] and later to *Ceratocystis* by Moreau [52]. It has since been referred back to *Ophiostoma* by de Hoog & Scheffer [41], who distinguished *Ophiostoma* from *Ceratocystis*. The *Graphium* conidial stage was referred to *Pesotum* by Crane & Schoknecht [32], while the mycelial conidia have been ascribed to *Sporothrix* by Hunt [43]. The latter was further described by de Hoog [40].

Research following the occurrence of the second pandemic in Britain in the early 1970s revealed that two distinct forms of the fungus were present in both Europe and North America. The two forms were initially termed the aggressive and non-aggressive strains [37], and later the aggressive and non-aggressive subgroups of the pathogen [10, 11]. The non-aggressive, a more weakly pathogenic fungus, is now believed responsible for the first pandemic of the disease [13, 19, 39] in Europe and North America in the 1920s–1940s. The highly pathogenic aggressive subgroup is responsible for the current second pandemic of the disease [13, 19, 39]. The aggressive subgroup was itself later shown to comprise two genetic entities, the Eurasian or EAN and North American or NAN races, with initially very different geographical distributions [8, 13, 15,-19, 22].

As comparative information on the aggressive and non-aggressive subgroups has accumulated, the extent of the biological differences between them has become more apparent (see Table 1). They have characteristic colony appearances, different growth rates and strikingly different temperature optima. Not only is the non-aggressive a weak and the aggressive a strong pathogen on elms of moderate resistance and in elm bark, but they produce contrasting levels of the protein toxin cerato-ulmin and show different cell wall degrading ability. They differ in the frequency of the two sexual compatibility types in nature, and in protoperithecial production and fecundity. They also differ in the structure and intensity of their vegetative incompatibility interactions.

Of particular significance is the fact that a unidirectional reproductive barrier occurs between the two subgroups, in that the aggressive as female strongly rejects the non-aggressive as male mating partner [6, 7, 14, 48]. Moreover, in aggressive × non-aggressive crosses the hybrids are of generally low competitive fitness. Thus F_1 hybrids typically show a remarkable range of non-parental colony patterns, and exhibit negative interactions with resulting low fitness for characters such as pathogenicity, growth-rate and cerato-ulmin production. Many hybrids are also female sterile [6, 10, 16, 18, 26, 48]. This indicates that the genomes of the aggressive and non-aggressive subgroups are incompatible as a consequence of independent evolution. In addition such hybrids have not been observed at current epidemic fronts even though the aggressive subgroup is constantly migrating into areas previously occupied only the non-aggressive. Some apparently rare introgression from non-aggressive to aggressive may occur [2, 48], but the distinctive biological characteristics of the aggressive subgroup are maintained, and the non-aggressive subgroup is rapidly replaced by the aggressive in a manner resembling a species–species competitive interaction [13, 18, 19, 50].

By the early 1980s, the known level of biological differences between the aggressive and non-aggressive subgroups was such that they were already considered to be at least equivalent to subspecies [8, 11 28]. Although the use of the informal 'subgroup' terminology was continued, it was felt to be both clumsy and unsatisfactory. However, the option of formal recognition at the subspecies level was deferred partly because of the consequences for the wider literature on Dutch elm disease, and partly because it was considered that recognition at the species level might be more appropriate [22]. Recent molecular evidence has strengthened the latter view. The two subgroups differ considerably in their buffer soluble protein profiles [44, 47], and exhibit characteristic banding patterns for five enzymes [5].

Their nuclear DNA patterns revealed by restriction enzymes are highly distinctive [2, 4], with approximately 75% of probes with random genomic DNA clones yielding distinguishing polymorphisms [2]. Their mitochondial DNA patterns are also distinctive [2, 4, 46], and the mtDNA genome of the non-aggressive subgroup is larger than that of the aggressive [2, 3, 46]. These differences are similar to those shown between morphospecies and biological species in other fungal groups [30, 34, 35, 60, 64].

Perithecial dimensions

In view of the above differences the perithecial dimensions of the aggressive and non-aggressive subgroups were re-investigated. Buisman [31] originally described *O. ulmi* as having perithecia with bases of 105–135 μm diameter and necks of 265–380 μm length. Buisman's herbarium material is unfortunately believed lost (de Hoog [40] and G.S. de Hoog personal communication). However, Buisman's original parameters have remained largely unmodified by later authors. Hunt [43] gives the perithecial base width as up to 135 μm and the perithecial neck length as 180–360 μm; while Uphadyhay [66] gives 110–160 (−185) μm and 100–300 (−350) μm respectively for the same criteria.

The perithecial dimensions of a wide geographical range of isolates of both subgroups were examined on elm twigs, the substrate used by Buisman, and on elm sapwood agar (Table 2). On elm twigs the two subgroups differed in their gross perithecial dimensions; in particular in their range of neck lengths and neck length : base width ratios. The dimensions of the non-aggressive subgroup conform fairly closely to those given by Buisman and others for *O. ulmi* [31, 43, 66], although the neck lengths at up to 420 (−513) μm are a little longer than previously reported. The neck lengths of the aggressive subgroup, however, while similar to the non-aggressive at the lower end of the size range, are considerably larger than those previously reported at up to 640 (−1073) μm. This difference is also reflected in the higher neck length : base width ratios of the aggressive isolates (Table 2).

The aggressive subgroup does not, therefore, conform to the existing concept of *O. ulmi* as a species which 'differs from other species with a Graphium state in having small perithecia with short necks' (Hunt [43]) or as a species with 'necks usually less than 360 μm long' (Upadhyay [66]). This feature of the aggressive subgroup is largely due to the EAN race, which often has longer necks than the NAN (to be published elsewhere). It results in a bimodal distribution of neck lengths within the aggressive subgroup which precludes a simple aggressive versus non-aggressive statistical comparison.

The perithecial bases of both subgroups tended to be larger on elm sapwood agar than on elm twigs, while in the non-aggressive the necks also tended to be shorter than on twigs (Table 2). In consequence there was a smaller overlap in the neck length-base width ratios of the two subgroups. On both substrata the perithecial neck widths and ostiolar hyphal lengths of the two subgroups were similar to those reported by Buisman and others [31, 43, 66]. However, although Buisman [31] described *O. ulmi* as having perithecial bases 'mostly with a few scattered hairs', in the present study the perithecia of the non-aggressive were occasionally, and those of the aggressive more often, moderately to densely bristly. The lengths of these bristles in the two subgroups were similar at up to c. 130 × 3 μm.

Status of the aggressive and non-aggressive taxa

The aggressive and non-aggressive subgroups, which are distributed across much of the Northern Hemisphere [13, 19, 22], not only show unique combinations of a wide range of morphological, physiological and genetical characters, but posess different nuclear and mitochondrial DNA architecture, and exhibit strong reproductive isolation at both the pre- and post-zygotic levels. It is considered that the differences de-

Table 2. Perithecial dimensions of the non-aggressive and aggressive subgroups

	Non-aggressive common range			Aggressive (EAN + NAN races) common range		
On elm twigs						
Neck length (NL) μm	(233–)	280–420	(–513)	(168–)	230–640	(–1073)
Base width (BW) μm	(84–)	100–150	(–177)		75–140	(–159)
NL:BW ratio	(1.4–)	2.4–3.5	(–4.0)		1.5–6.2	(–8.3)
On elm sapwood agar						
Neck length (NL) μm	(177–)	190–350	(–429)	(224–)	250–600	(–700)
Base width (BW) μm	(93–)	120–180	(–187)	(103–)	130–180	(–187)
NL:BW ratio		1.2–2.3	(–2.5)	(1.4–)	1.7–3.9	(–4.8)

Figures given are common ranges with extremes in parentheses

Elm twig data shown are for a total of 90 non-aggressive and 149 aggressive perithecia (79 EAN + 70 NAN). Ten mature, oozing, perithecia were taken at random for each of 18 within country and 6 between country A-type × B-type pairings as follows. Non-aggressive pairings: P32 × P98 (Poland), TR116 × TR65 (Turkey), H173 × H877 (USA), PG401 × PG386 (Portugal), Yu99 × Yu38 (Yugoslavia), GolB4 × P98 (Iran and Poland), H173 × I185 (USA and Italy), GolB4 × TR118 (Iran and Turkey), PG401 × H200 (Portugal and Ireland). Agressive pairings: EAN, H581 × H582 (Iran), CA1 × CA4 (Tashkent, USSR), R64 × R103 (Romania), H236 × H237 (Germany), H327 × H323 (Czechoslovakia), P127 × P155 (Poland), Yu2 × Yu1 (Yugoslavia), Yu16 × CKT-11 (Yugoslavia and Iran); NAN, ES1343 × ES122 (Spain), T259 × T255 (UK), H351 × H352 (Belgium), H2117 × H2118 (USA), H249 × H250 (The Netherlands), H2091 × H161 (USA), MM2/1 × H363 (UK and Ireland). The pairings were made 2 cm apart on 10 × 0.5 cm autoclaved split peeled elm twigs following the method of Tchernoff [65]. The inoculated twigs were kept in test tubes, moistened with 1 ml sterile water, and incubated 7 days at 15°C followed by 10–14 days in diffuse light 20–23 °C.

Elm sapwood agar data shown are for a total of 49 non-aggressive and 56 aggressive subgroup perithecia. The recipients (♀) were inoculated singly to ESA [9] plates and incubated at 20 °C (30 °C for non-aggressive isolates) in darkness for 7 days and then in diffuse light at 20–23 °C for 5 days. They were then fertilised with conidia (♂) of a mating type compatible donor isolate as described in Ref 9. After a further 10–14 days incubation at 20–23 °C six to eight mature, oozing, perithecia were removed at random for each pairing. Isolate numbers and geographical origins of the recipient isolates were: non-aggressive × non-aggressive, GolB4 (Iran), TR116 (Turkey), P98 (Poland), H830 (USA), PG401, (Portugal), H411 (Ireland), H173 (USA), TR118 (Turkey); EAN × EAN, Yu16, (Yugoslavia), CA1 (Tashkent, USSR), P127 (Poland), H322 (Czechoslovakia) NAN × NAN, MM2/1 (UK), H670 (USA), H172 (USA), H351 (Belgium). All recipients were A-types except P98 and TR118 which were B-types.

monstrated between them are now such that they should be recognised as distinct though closely related species of *Ophiostoma*. The 'old' non-aggressive subgroup described by Buisman [31] is therefore retained as *O. ulmi* and the aggressive subgroup proposed as a new species, *O. novo-ulmi*. The descriptions of the two species as now interpreted are given below. This reclassification has an additional advantage in that it puts the historical and biological role of the non-aggressive during the first Dutch elm disease epidemic in the early part of this century in its rightful perspective. This is particularly pertinent because the non-aggressive may be in danger of extinction as a result of its continuing replacement by the aggressive [13, 19]. It also emphasises the need to consider the possibility of separate geographical origins for the two taxa [22].

***Ophiostoma novo-ulmi* Brasier sp. nov.**

Similis Ophiostomae ulmi (Buism.) Nannf. *sed differt in rostro peritheciis circa 230–640 (– 1070) μm altis; et in cultura in extracto malti cum temperatura crescenti optime circa 20–22°C et maxime circa 33°C, et cum coloniis fibroso-striatis plerumque plus 3.1–4.8 (–5.5) mm radiis per diem ad 20°C crescentibus.*

Holotypus IMI 343091: a dried culture on elm wood of paired isolates IMI 343092 × IMI 343097 Isotypes CBS 435.90, DAOM 211971. IMI 343092 is an EAN race, A-compatibility type isolate from xylem of *Ulmus carpinifolia* collected at Swinoujscie, Poland by C.M. Brasier in August 1980; IMI 343097 is an EAN race, B-compati-

bility type isolate from xylem of *U. carpinifolia* collected at Szczecin, Poland by C.M. Brasier in August 1980.

Colonies on malt extract agar [19] after 7 days in darkness at 20° and 10 days in diffuse daylight greyish-white to cream-white ranging from regular striate petaloid forms to irregular lobed forms; commonly with moderate aerial mycelium aggregated into ropes to give a fibrous striate appearance or occasionally with less aerial mycelium and frosty to smooth colonies. Diurnal zonation moderate to strong. Growth on malt extract agar at 20° in darkness ranging from (2.8–) 3.1–4.8 (−5.7) mm day^{-1}; growth optimum c. 20–22°; maximum 32–33°. *Note:* colonies may become felty to dense woolly or slow growing degenerate-'amoeboid' looking due to virus associated disease or to degeneration during storage [11, 23].

Hyphae septate, c. 1–6 μm diam, submerged hyphae sometimes up to 10 μm diam; aerial hyphae often aggregated into strands. Mycelial conidia usually abundant, *Sporothrix*: conidiophores mostly lateral, c. 10–30 (−50) μm; conidia holoblastic, borne on short denticles of c. 0.5–1 μm, single celled, hyaline, very variable ellipsoid to elongate, often tapering and slightly curved, with a small attachment collar, 4.5–14 × 2–3 μm. Mycelial conidia often aggregated into mucilaginous droplets, also budding in a yeast-like fashion. Synnematal anamorph (*Graphium* or *Pesotum*) usually absent on malt agar, generally produced only on sterilised elm sapwood (but abnormal synnemata may be produced on malt agar by degenerate colonies); single or multiple, brown-black, slender, up to 1–2 mm tall. Attached to substratum by brown rhizoid-like hyphae and composed of parallel bundles of brown septate hyphae, flaring at the top to branched hyaline hyphae producing holoblastic single-celled hyaline ovoid to ellipsoid conidia c. 2–6 × 1–3 μm, aggregating into a cream-white mucilaginous spore drop. The holoblastic budding yeast-like anamorph is produced in liquid cultures, and on the surfaces of solid media.

Heterothallic with two compatibility types, 'A' and 'B'. A-types producing brown-black protoperithecia, occasional to frequent on malt agar and frequent to abundant on elm sapwood agar. B-types producing ascogonia sporadically on malt agar and ascogonia or protoperithecia occasionally to frequently on elm sapwood agar. Perithecia, superficial to partially immersed, attached to the substratum by brown rhizoid-like hyphae; the base globose, black, 75–140 μm wide, sparsely to moderately bristly, the bristles brown-black, septate, measuring up to 130 × 3 μm; the necks black, 230–640 (−1070) μm long, 19–36 μm diam at base, 9–14 μm at tip; neck length/base width ratio commonly 1.5–6.2; ostiolar hyphae numerous, hyaline septate, rarely branched, c. 20–60 μm × 1–2 μm; tips sometimes producing conidia. Asci thin walled, globose to oval, evanescent. Ascospores hyaline, single-celled, orange segment shaped, c. 4.5–6 × 1–1.5 μm, accumulating as a cream-white mucilaginous spore drop. B-compatibility type usually predominant in natural populations, though less so in North America. As the female (i.e. protoperithecial) mating partner, strongly rejects *O. ulmi* as male mating partner. Cerato-ulmin protein production by healthy iolates in liquid cultures abundant. Mitochondrial DNA and nuclear DNA polymorphism patterns different from those of *O. ulmi*. Mitochondrial DNA size c. 48–71 kb (Table 1).

Pathogenicity. Strong on 2 m tall *Ulmus procera* causing c. 60–100% defoliation, with recovery rare; and weak to moderate on 4 m tall *U. x* 'Commelin' causing c. (20–) 45–90% defoliation (Table 1).

Habitat. In discoloured xylem and in the bark of elms (*Ulmus* spp); particularly in and around breeding galleries of vector scolytid beetles; usually as a causal agent of the vascular wilt disease of elms known as Dutch elm disease.

Distribution: Widely distributed in North America, across Europe including European Russia

and the Ukraine; and in Turkey, the Caucasus, Iran and Uzbekistan [22].

Living cultures IMI 343092 – IMI 343111. To reduce the risk of degeneration [e.g. see Ref. 11, *Fig. 7* and Ref. 9, *Fig. 103*] liquid nitrogen storage of young portions of healthy cultures exhibiting 'wild-type' colony morphology [e.g. Ref. 9, *Fig. 104*] is recommended.

Having described *O. novo-ulmi* it becomes necessary to redefine the concept of *O. ulmi*. In view of the fact that there is no record of a type for *O. ulmi* being designated and no authentic material of *O. ulmi* is believed available, a neotype of this species is designated.

Ophiostoma ulmi (Buism.) Nannf. [31, 49] syn. *Ceratocystis ulmi* (Buism.) Moreau [31, 52]

Colonies on malt extract agar [19] after 7 days in darkness at 20° and 10 days in diffuse daylight smooth waxy to lawns of relatively undifferentiated or delicately striate aerial mycelium. Diurnal zonation usually weak to moderate. Colonies creamy-white to yellow-brown sometimes with purple or brown patches. Pigmented or non-pigmented sectors common. Growth on malt extract agar at 20° ranging from (1.5–) 2.0–3.1 (–3.5) mm day^{-1}; growth optimum (25–) 27.5–30°; maximum c. 35°. *Note:* colonies may become very slow, irregular and/or dense grey-white felty to woolly due to virus associated disease or to degeneration during storage (11, 23).

Hyphae septate, c. 1–6 μm diam, submerged hyphae sometimes to 10 μm wide; aerial hyphae often aggregated into strands. Mycelial conidia abundant, *Sporothrix*: conidiophores mostly lateral, c. 10–30 (–50) μm; conidia holoblastic, borne on short denticles of c. 0.5–1 μm; single-celled, hyaline, very variable, ellipsoid to elongate, often tapering and slightly curved, with a small attachment collar, 4.5–14 × 2–3 μm. Mycelial conidia often aggregated into mucilaginous droplets, also budding in a yeast-like fashion; aggregates of the mycelial conidia and budding conidia often coalescing to a yeast-like mass, conferring a waxy appearance to the colonies. Synnematal anamorph (*Graphium* or *Pesotum*) usually absent on malt agar generally produced only on sterilised elm sapwood (but abnormal synnemata may be produced on malt agar by degenerate colonies); single or multiple, brown-black, slender, up to 1–2 mm tall. Attached to substratum by brown rhizoid-like hyphae and composed of parallel bundles of brown septate hyphae, flaring at the top to branched hyaline hyphae producing holoblastic single-celled hyaline ovoid to ellipsoid conidia c. 2–6 × 1–3 μm, aggregating into a cream-white mucilaginous spore drop. The holoblastic budding yeast-like anamorph is produced in liquid cultures, and on the surfaces of solid media.

Heterothallic with two compatibility types, 'A' and 'B'. Ascogonia usually absent on malt agar; protoperithecial production often sporadic (occasionally plentiful) on elm sapwood agar. Perithecia, superficial to partially immersed, attached to the substratum by brown rhizoid-like hyphae; the base globose, black 100–150 μm wide, sparsely to moderately bristly, the bristles brown-black, septate, measuring up to 130 × 3 μm; the necks black, 280–420 (–510) μm long, 18–42 μm diam at base, 11–16 μm at tip; neck length/base width ratio commonly 2.4–3.5; ostiolar hyphae numerous, hyaline septate, rarely branched, c. 20–60 μm × 1–2 μm, tips sometimes producing conidia. Asci thin walled globose to oval, evanescent. Ascospores hyaline, single-celled, orange segment shaped, c. 4.5–6 × 1–1.5 μm, accumulating as a cream-white mucilaginous spore drop. A and B compatibility types occurring in about equal frequency in natural populations, or (North America) the A-type predominates. As the female (ascogonial) partner, accepts *O. novo-ulmi* as the male mating partner. Cerato-ulmin protein production by healthy isolates in liquid cultures nil to very low. Mitochondrial DNA and nuclear DNA polymorphism patterns rather different from those of *O. novo-ulmi*. Mitochondrial DNA size c. 74–88 kb.

Pathogenicity. Weak on 2 m tall *Ulmus* procera,

Table 3. Differences between the EAN and NAN races of *O. novo-ulmi* sp. nov.

	EAN race	NAN race	References
Morphological			
Perithecial neck length (elm twigs)	Often longer	Often shorter	To be published separately
Colony morphology at 20 °C	——— Some differences ———		8, 9, 11, 15
Physiological			
Radial growth rate (mm/day^{-1}) of wild-type isolates at 20 °	3.1–4.4*	3.2–4.8	8, 9, 11, 15, 27
Pathogenicity (% defoliation)			9, 10, 12, 15, 19
on clonal *U. procera*	(40–)60–100%	80–100%	
on *U.* ×'Commelin'	(20–)40–90%	60–20%	
'*Up-mut*' colony dimorphism	Present	Absent	10, 15, 18, 19, 20
Biomass production in liquid cultures	Higher	Lower	29, 45
Cerato-ulmin production in liquid cultures	Lower	Higher	29, 45
Fertility reaction as ♀	Partially rejects NAN	Accepts EAN	8, 9, 14, 18
Molecular			
Protein and isozyme polymorphisms	——— Some differences ———		45
Mitochondrial DNA size	65–71 kb	48–60 kb	2, 3
Mitochondrial DNA polymorphisms	——— Distinctive ———		2, 4
Nuclear DNA polymorphisms	——— Characteristic differences ———		2, 4

Ranges given are common ranges only and frequencies given are 'norms'. For extremes see references.
* *N.B.* The '*up-mut*' colony form of the EAN may grow more slowly [15]. ESA = elm sapwood agar [9].

causing *c.* (2–)10–35(–40)% defoliation followed by recovery. No external symptoms on 4 m tall *U.* × 'Commelin'.

Habitat. In discoloured xylem and in the bark of elms (*Ulmus* spp.); particularly in and around breeding galleries of scolytid vector beetles; usually as a causal agent of the vascular wilt disease of elms known as Dutch elm disease:

Distribution. Widely distributed in North America, across Europe including European Russia and the Ukraine; and in Turkey, the Caucasus, Iran and Uzbekistan [22].

Neotype IMI 343078: a dried culture on elm wood of paired isolates IMI 343079 × IMI 343085. Isoneotypes CBS 436.90 and DAOM 211970. IMI 313079 is an A-compatibility type isolate from bark of *Ulmus glabra* collected at Golestan Forest, Gorgan, Iran by C.M. Brasier and F.A. Afsharpour in October 1977; IMI 343085 is a B-compatibility type isolate from xylem of *U. carpinifolia* collected at Pammakule, southern Turkey by C.M. Brasier in October 1980.
Living cultures IMI 343079-IMI 343090. As with *O. novo-ulmi* (see above), liquid nitrogen storage of young portions of healthy cultures in 'wild-type' condition is recommended to reduce the risk of degeneration.

The differences between *O. ulmi* and *O. novo-ulmi* are summarised in Table 1.

Status of the EAN and NAN races of *O. novo-ulmi*

The EAN and NAN races of *O. novo-ulmi* differ in a number of key biological characters, and a partial fertility barrier operates between them (Table 3). Taking these differences into account, the EAN and NAN were originally designated races in the broad biological sense [8, 10, 11]. In contrast to the *O. ulmi* × *O. novo-ulmi* interaction in the field, where no independent hybrids have appeared, EAN × NAN hybrids are now appearing widely in western Europe where their

ranges have overlapped [17]. Indeed, EAN/NAN hybrid-swarms could become the dominant form of *O. novo-ulmi* at many western European locations. In western Europe therefore, the *O. novo-ulmi* complex is undergoing rapid evolution. Equally however, the EAN and NAN may continue to remain discrete entities at locations geographically isolated or more distant from western Europe, such as North America where only the NAN race is known; and from central Europe through to central Asia where only the EAN race is known. It may therefore be appropriate to give the EAN and NAN formal recognition at the subspecies level. The status of the EAN and NAN is currently under investigation, and will be dealt with in a separate publication.

A single isolate of *Ophiostoma* from elm in the Himalayas shows a range of characteristics distinct from that of both *O. ulmi* and *O. novo-ulmi* [12, 22] and may be another species. Resolution of its status should be delayed until further Himalayan material has been examined.

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