European Journal of Plant Pathology **106:** 907–912, 2000.

Short communication

Fusarium redolens f.sp. *asparagi*, causal agent of asparagus root rot, crown rot and spear rot

R.P. Baayen[1], P.H.J.F. van den Boogert[2], P.J.M. Bonants[2], J.T.K. Poll[3], W.J. Blok[4] and C. Waalwijk[1]
[1]*Plant Protection Service, Mycology Section, P.O. Box 9102, 6700 HC Wageningen, The Netherlands (Phone: 31 317 496830; Fax: +31 317 421701; E-mail: r.p.baayen@pd.agro.nl);* [2]*Plant Research International, Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands;* [3]*Applied Research for Arable Farming and Field Production of Vegetables, P.O. Box 430, 8200 AK Lelystad, The Netherlands;* [4]*Biological Farming Systems Group, Wageningen University and Research, Marijkeweg 22, 6709 PG Wageningen, The Netherlands*

Accepted 7 September 2000

Key words: Asparagus officinalis, Fusarium oxysporum, Fusarium proliferatum, Fusarium redolens

Abstract

Two *Fusarium* species, *F. oxysporum* f.sp. *asparagi* and *F. proliferatum*, are known to be involved in the root and crown rot complex of asparagus. We have investigated reports on the involvement of *F. redolens*, a third species, which until recently was considered conspecific with *F. oxysporum* because of morphological similarities. RFLP analysis of the rDNA internal transcribed spacer region and AFLP fingerprinting identified eight strains from asparagus unambiguously as *F. redolens*. Four of these were tested and found to be pathogenic to asparagus either in this study (two strains) or in a previous one in which they were classified as *F. oxysporum* (three strains). Disease symptoms and disease development were the same as with *F. oxysporum* f.sp. *asparagi* and *F. proliferatum*. Present data and literature reports identify *F. redolens* as a host-specific pathogen involved in root, crown and spear rot of asparagus. The pathogen is formally classified as *F. redolens* Wollenw. f.sp. *asparagi* Baayen.

Several pathogenic *Fusarium* species are associated with asparagus diseases. Foot and stem rot is caused by *F. culmorum* (W.G. Smith) Sacc., while root and crown rot are generally ascribed to *F. oxysporum* Schlecht.:Fr. f.sp. *asparagi* Cohen & Heald and *F. proliferatum* (Matsushima) Nirenb. (Elmer, 1990, 1991, 1995; Elmer and Stephens, 1989; Blok and Bollen, 1995, 1997; Elena and Kranias, 1996). Spear rot of asparagus has been attributed to *F. redolens* Wollenw., a species that has also been reported to be associated with root and crown rot of asparagus (Gerlach, 1961; UnterEcker, 1972; Cheah, 1986; Falloon and Tate, 1986; Gordon-Lennox and Gindrat, 1987; Sadowski and Knaflewski, 1990).

Fusarium redolens was described by Wollenweber (1913) and maintained in his following publications (Wollenweber, 1916–1935, 1931; Wollenweber and Reinking, 1935). Outside Germany, the country where Wollenweber's taxonomic system was followed (Gerlach and Nirenberg, 1982), *F. redolens* has long been considered conspecific with *F. oxysporum* (Snyder and Hansen, 1940; Nelson et al., 1983) or at best a variety of this species, *F. oxysporum* Schlecht.:Fr. var. *redolens* (Wollenw.) Gordon (Gordon, 1952; Booth, 1971, 1975). Morphological distinction of *F. redolens* from *F. oxysporum* is indeed problematic due to the presence of intermediate forms (Baayen and Gams, 1988). Unambiguous distinction between both species has only recently become possible through molecular means such as RFLP analysis of the rDNA internal transcribed spacer (ITS) region and gene sequence analysis (Waalwijk et al., 1996a,b; O'Donnell et al., 1998). As a consequence, it is uncertain whether former reports on the involvement

of *F. redolens* in asparagus rot are reliable, and also, whether published reports on *F. oxysporum* f.sp. *asparagi* pertain to this taxon, or to *F. redolens*, or to members of both species.

The involvement of *F. redolens* in the spear, root and crown rot complex of asparagus was evaluated in the present study using ITS-RFLP and AFLP analyses as well as pathogenicity tests.

Identification of strains as *Fusarium redolens*

Isolates from asparagus received as *F. oxysporum* and/or *F. redolens* were obtained from The Netherlands, Germany, Poland, Italy, Greece, U.S.A., South Africa, and New Zealand. Among these were members of 17 vegetative compatibility groups (VCG 1–9 and VCG 11–18) of *F. oxysporum* f.sp. *asparagi* present in The Netherlands (Blok and Bollen, 1997), and members of *F. oxysporum* f.sp. *asparagi* VCG 1001–1008 present in the U.S.A. (Elmer and Stephens, 1989). In total 126 strains were examined. Additionally, three strains of *F. proliferatum* were selected as a reference.

Fungal strains were grown in potato dextrose broth (Difco) at 25 °C for 3 days. DNA was isolated from lyophilized mycelium as reported previously (Waalwijk et al., 1996a). The ITS region was amplified with primers ITS-1 and ITS-4, and amplification products were digested individually with *Hinf* I, *Alu*I, *Mun*I, and *Rsa*I and analyzed on 1.5% agarose gels as reported previously (Waalwijk et al., 1996a). Isolates were identified by their characteristic ITS restriction fragment length profile (Table 1). ITS-RFLP profiles characteristic of *F. redolens* were obtained for eight strains from The Netherlands, Germany and Poland (Table 2); 117 of the remaining 118 strains were classified as *F. oxysporum* (not shown). Strain IPO 97-10 from New Zealand, received as *F. redolens* under the code 4869-95, proved to belong to *F. proliferatum* and indeed

Table 1. Restriction fragment length sizes of amplified DNA from the ITS region of *F. oxysporum*, *F. redolens* and *F. proliferatum* restricted with *Hinf* I, *Alu*I, *Mun*I, and *Rsa*I

	Hinf I	*Alu*I	*Mun*I	*Rsa*I
F. oxysporum	90, 180, 265	60, 135, 350	545	545
F. redolens	265, 290	560	560	560
F. proliferatum	265, 290	140, 420	70, 490	100, 460

Table 2. Strains of *F. redolens*, *F. oxysporum* and *F. proliferatum* from asparagus plants or from asparagus production field soil. Strains found to belong to *F. oxysporum* and exclusively tested by ITS-RFLP are not listed

Isolate	Origin	Source	ITS-RFLP*	AFLP*	Bioassay*
F. redolens					
CWB1	The Netherlands	W.J. Blok	+	+	+
CWB33	The Netherlands	W.J. Blok	+	+	−
CWB42	The Netherlands	W.J. Blok	+	+	−
IPO 97-13	The Netherlands	J.T.K. Poll	+	+	+
IPO 97-143	The Netherlands	R.P. Baayen	+	−	−
IPO 97-158	The Netherlands	R.P. Baayen	+	+	−
DSM 62380	Germany	W. Gerlach	+	+	−
KFL63	Poznan, Poland	H. Kwasna	+	−	−
F. oxysporum					
MA25 (= FGSC 6607)	U.S.A.	W.H. Elmer	+	+	−
IPO 97-12	The Netherlands	J.T.K. Poll	+	+	−
IPO 97-14	The Netherlands	J.T.K. Poll	+	−	+
IPO 97-102	The Netherlands	R.P. Baayen	+	−	+
IPO 97-147	The Netherlands	R.P. Baayen	+	+	−
IPO 97-172	The Netherlands	R.P. Baayen	+	+	−
F. proliferatum					
IPO 97-03	The Netherlands	J.T.K. Poll	+	+	−
IPO 97-10	New Zealand	E.H.C. McKenzie	+	+	+
M6371	U.S.A.	W.H. Elmer	+	+	−
IPO 97-101	The Netherlands	R.P. Baayen	+	+	+

*Included (+) or not included (−) in the analyses.

was found to produce the characteristic microconidial chains of this species. Three strains presently identified as *F. redolens* (CWB1, CWB33 and CWB42) had previously been considered to belong to *F. oxysporum* f.sp. *asparagi* (Blok and Bollen, 1997) and represent the three main vegetative compatibility groups (VCG 1, VCG 9 and VCG 12) found in the latter study among 24 isolates from asparagus in The Netherlands.

Six isolates of *F. redolens* identified as such by ITS-RFLP were further characterized by AFLP fingerprinting, along with four isolates each of *F. oxysporum* and *F. proliferatum*. AFLP analyses were performed as described previously (Baayen et al., 2000). Briefly, DNA was digested with *Eco*RI and *Msp*I, followed by ligation of adapters and non-selective amplification with zero primers *Eco*00 (5′GACTGCGTACCAATTC) and *Msp*00 (5′GATGAGTCCTGATCGG). Selective PCR was performed on the amplification product with Cy5-labelled fluorescent *Eco*20 primer (5′GACTGCGTACCAATTCGC) and either *Msp*15 (5′GATGAGTCCTGATCGGCA) or *Msp*16 (5′GATGAGTCCTGATCGGCC) primer. Denatured product was loaded on 6% polyacrylamide gels and run on the automatic sequencer ALFexpress (Pharmacia) at 1500 V, 60 mA, 25 W and 55 °C. A fluorescently labelled 50 bp ladder (Pharmacia) was used as a standard. AFLP patterns were analyzed with ImageMaster software (Amersham Pharmacia Biotech). Only reproducible bands were incorporated in the analysis. A similarity matrix was constructed using the method of Nei and Li (1979). UPGMA cluster analysis of binary data was performed with Treecon software (Van de Peer and De Wachter, 1994). UPGMA similarity analysis supported the distinction of *F. redolens* as a species distinct from *F. oxysporum* and *F. proliferatum* with 100% bootstrap support (Figure 1).

Pathogenicity of *Fusarium redolens* to asparagus

The pathogenicity of CWB1, formerly considered to belong to *F. oxysporum* f.sp. *asparagi* but presently shown to belong to *F. redolens*, is well known (Blok and Bollen, 1997). CWB1 is pathogenic to asparagus, but not to 20 other plant species including *Allium* spp., *Anethum graveolens*, *Apium graveolens*, *Beta vulgaris*, *Brassica oleracea*, *Chenopodium album*, *Daucus carota*, *Linum usitatissimum*, *Lolium perenne*, *Medicago sativa*, *Phaseolus vulgaris*, *Poa annua*, *Stellaria media*, *Vicia faba*, and *Zea mays*. Occasionally, pea and lupin may show mild symptoms of foot rot and root rot with CWB1. The same pattern of host specificity was observed for strain CWB 7 (Blok and Bollen, 1997) of which the classification as *F. oxysporum* f.sp. *asparagi* was presently confirmed by ITS-RFLP. The above-mentioned reports by Blok and Bollen (1997) on the host specificity of *F. redolens* CWB1 are concordant with earlier reports by Graham (1955) for *F. redolens* from asparagus. Both *F. oxysporum* f.sp. *asparagi* and *F. redolens* from asparagus thus appear to be restricted in pathogenicity to asparagus.

Apart from its possible involvement in the asparagus rot complex, *F. redolens* has been reported to be responsible for rot and wilt diseases in, among

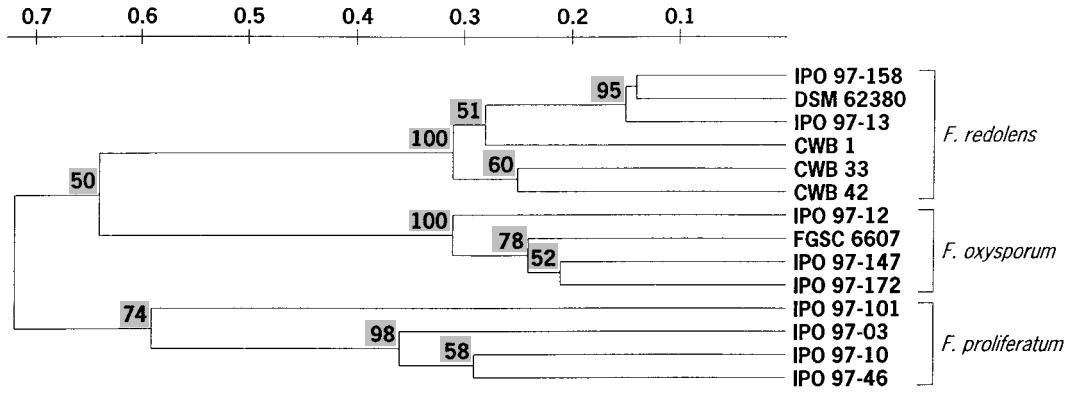

Figure 1. UPGMA similarity analysis of six strains of *F. redolens* from asparagus, and four strains each of *F. oxysporum* f.sp. *asparagi* and *F. proliferatum*. Dissimilarity percentages between isolates are given on the *X*-axis; bootstrap support percentages (1000 replicates) are indicated at the nodes.

others, carnation, conifers, flax, pea, and spinach (Gerlach and Pag, 1961). In several of these diseases the *F. redolens* strains involved are also host specific. Two formae speciales have been formally published, *F. redolens* f.sp. *dianthi* (Gerlach and Pag, 1961; Baayen et al., 1997, 1999) and *F. redolens* f.sp. *spinaciae* (Sherb.) Subramanian (Subramanian, 1971). Isolates of *F. redolens* f.sp. *dianthi* are not pathogenic to asparagus, and exclusively infect *Dianthus* species (Gerlach and Pag, 1961). The pattern of host specificity of *F. redolens* f.sp. *spinaciae* is not well documented in the original description (Hungerford, 1923).

Pathogenicity of *F. redolens* CWB1 to asparagus was confirmed in the present study for asparagus seedlings (cv. Gijnlim) grown in a growth chamber on water agar in culture tubes at 23 °C and 16 h illumination and inoculated 14 days after germination with a 1 × 1 mm agar piece from a Petri dish culture per seedling as described previously (Blok and Bollen, 1997). After 30 days, CWB1 was equally pathogenic to asparagus as *F. oxysporum* f.sp. *asparagi* IPO 97-102 and *F. proliferatum* IPO 97-101 (Figure 2a), with identical root infection symptoms. Noninoculated control plants remained entirely healthy. With a second isolate (IPO 97-13), similar results were obtained in a greenhouse test on cv. Thielim plantlets grown from disinfected seed in vermiculite in 200 ml Fibracan polystyrene pots at 20 °C and inoculated after 20 days with 10 ml conidial suspension (10^4 conidia ml^{-1}). After 30 days, shoots had developed disease symptoms with all three *Fusarium* species, provided that the roots had been damaged at inoculation by cutting off part of the roots (Figures 2b and c).

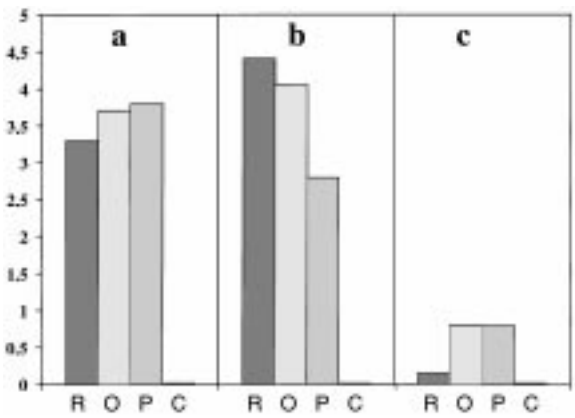

Figure 2. Average disease index of asparagus seedlings, 30 days after inoculation with *F. redolens* (R), *F. oxysporum* (O), or *F. proliferatum* (P). C, noninoculated control. (a) Asparagus cv. Gijnlim grown in tubes with water agar and inoculated with strains CWB1 (*F. redolens*), IPO 97–102 (*F. oxysporum*) or IPO 97–101 (*F. proliferatum*). Root damage was assessed on an ordinal scale from 0 (unaffected) to 5 (completely rotten and hollowed-out; shoots also dead). (b) Asparagus cv. Thielim grown in pots with vermiculite and inoculated with strains IPO 97–13 (*F. redolens*), IPO 97–14 (*F. oxysporum*) or IPO 97–10 (*F. proliferatum*). Root system wounded at inoculation. Shoot damage was assessed on an ordinal scale from 0 (healthy plants) to 5 (dead plants). (c) As in B but root system not wounded at inoculation.

Table 3. Origin of 86 isolates of *F. oxysporum*, *F. redolens* and *F. proliferatum* from asparagus from the Netherlands

	Roots	Spears
F. oxysporum	46	21
F. redolens	3	1
F. proliferatum	11	4

No support was obtained for the hypothesis of Cheah (1986) and Falloon and Tate (1986) that *F. redolens* is specifically associated with spear root, rather than root and crown rot (Table 3). Indeed, Gerlach and Pag (1961) had already reported *F. redolens* from diseased asparagus roots, rhizomes, crowns and stem base lesions. Similarly, UnterEcker (1972) reported *F. redolens* as a root rot pathogen of asparagus. Gordon-Lennox and Gindrat (1987) reported the abundance of *F. redolens* (as *F. oxysporum* var. *redolens*) on necrotic asparagus roots; isolates of *F. redolens* from such roots were all highly pathogenic to asparagus seedlings.

In conclusion, data from our experiments as well as from the literature convincingly show that *F. redolens* is a specialized pathogen of asparagus associated with root rot, crown rot and spear rot. In line with previous descriptions of host-specific forms of *F. redolens*, we propose the following name for the pathogen involved:

Fusarium redolens **Wollenw. f.sp.** ***asparagi*** **Baayen**

The fungus involved has been reported from Europe (present data), Canada (Gerlach and Pag, 1961), and New Zealand (Cheah, 1986; Falloon and Tate, 1986). Further investigations on strains from asparagus considered to belong to *F. oxysporum* may reveal a widespread occurrence of this fungus throughout asparagus production areas all over the world.

Acknowledgements

We are pleased to acknowledge the skilled laboratory assistance of Marieke Förch, Marianne Hagenaar-de Weerdt, and Jacq de Koning. Strains were generously made available by K. Elena, W.H. Elmer, H. Kwasna, S.C. Lamprecht, A. Logrieco and E.H.C. McKenzie. These investigations were supported in part by the Netherlands Ministry of Agriculture, Fisheries and Nature Management (programme DWK-337).

References

Baayen RP, van Dreven F, Krijger MC and Waalwijk C (1997) Genetic diversity in *Fusarium oxysporum* f.sp. *dianthi* and *Fusarium redolens* f.sp. *dianthi*. Eur J Plant Pathol 103: 395–408

Baayen RP and Gams W (1988) The *Elegans* fusaria causing wilt disease of carnation. I. Taxonomy. Neth J Plant Pathol 94: 273–278

Baayen RP, O'Donnell K, Bonants PJM, Cigelnik E, Kroon LPNM, Roebroeck EJA and Waalwijk C (2000) Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology 90: 891–900

Baayen RP, O'Donnell K, Waalwijk C, Bonants PJM, Cigelnik E and Roebroeck EJA (1999) Phylogeny of VCGs and formae speciales in *Fusarium oxysporum* and *F. redolens*. Phytoparasitica 27: 71–72

Blok WJ and Bollen GJ (1995) Fungi on roots and stem bases of asparagus in the Netherlands: species and pathogenicity. Eur J Plant Pathol 101: 15–24

Blok WJ and Bollen GJ (1997) Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum* f.sp. *asparagi*. Can J Bot 75: 383–393

Booth C (1971) The genus *Fusarium*. Commonwealth Mycological Institute, Kew, England

Booth C (1975) The present status of *Fusarium* taxonomy. Ann Rev Phytopathol 13: 83–93

Cheah LH (1986) Spear rot of asparagus. In: Proceedings 39th New Zealand Weed and Pest Control Conference (pp. 228–229) New Zealand Weed and Pest Control Society, Palmerston North, New Zealand

Elena K and Kranias L (1996) *Fusarium* spp. as a source of crown and root rot of asparagus in Greece. Bulletin OEPP 26: 407–411

Elmer WH (1990) *Fusarium proliferatum* as a causal agent in *Fusarium* crown and root rot of asparagus. Plant Disease 74: 938

Elmer WH (1991) Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and asparagus residue colonization among groups. Phytopathology 81: 852–857

Elmer WH (1995) A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. Mycologia 87: 68–71

Elmer WH and Stephens CT (1989) Classification of *Fusarium oxysporum* f.sp. *asparagi* into vegetatively compatible groups. Phytopathology 79: 88–93

Falloon PG and Tate KG (1986) Major diseases of asparagus in New Zealand. Proceedings Agronomy Society of New Zealand 16: 17–28

Gerlach W (1961) *Fusarium redolens* Wr., seine Morphologie und systematische Stellung. Ein Beitrag zur Kenntnis der *Elegans*-Fusarien. Phytopathol Zeitschr 42: 150–160

Gerlach W and Nirenberg HI (1982) The genus *Fusarium* – a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem 209: 1–406

Gerlach W and Pag H (1961) *Fusarium redolens* Wr., seine phytopathologische Bedeutung und eine an *Dianthus*-Arten gefäßparasitäre Form (*F. redolens* Wr. f. *dianthi* Gerlach). Phytopathol Zeitschr 42: 349–361

Gordon WL (1952) The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. Can J Bot 30: 236–238

Gordon-Lennox G and Gindrat D (1987) Relationship of root necrosis potential of soil to asparagus (*Asparagus officinalis*) decline in Switzerland. Biol Fertil Soils 3: 195–198

Graham KM (1955) Seedling blight, a fusarial disease of *Asparagus*. Can J Bot 33: 374–400

Hungerford CW (1923) Fusarium wilt of spinach. Phytopathology 13: 205–209

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Nat Acad Sci (USA) 76: 5269–5273

Nelson PE, Toussoun TA and Marasas WFO (1983) *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park, Pennsylvania, USA

O'Donnell K, Cigelnik E and Nirenberg HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90: 465–493

Sadowski C and Knaflewski M (1990) Susceptibility of selected asparagus cultivars to *Fusarium* spp. under field conditions. Acta Horticulturae 271: 343–351

Snyder WC and Hansen HN (1940) The species concept in *Fusarium*. Amer J Bot 27: 64–67

Subramanian CV (1971) Hyphomycetes. An account of Indian species, except Cercosporae. Indian Council for Agricultural Research, New Delhi

UnterEcker H (1972) Studies on infection by fusarium root rot of young asparagus plants in North Baden propagating holdings. Gesunde Pflanzen 24(6): 99–104

Van de Peer Y and De Wachter R (1994) Treecon for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Applic Biosci 10: 569–570

Waalwijk C, Baayen RP, de Koning JRA and Gams W (1996a) Ribosomal DNA analyses challenge the status of *Fusarium* sections *Liseola* and *Elegans*. Sydowia 48: 90–104

Waalwijk C, de Koning JRA, Baayen RP and Gams W (1996b) Discordant groupings of *Fusarium* spp. from sections *Elegans*,

Liseola and *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. Mycologia 88: 361–368

Wollenweber HW (1913) Studies on the *Fusarium* problem. Phytopathology 3: 24–50

Wollenweber HW (1916–1935). Fusaria autographice delineata. Berlin, 1200 plates

Wollenweber HW (1931) *Fusarium*-Monographie I. Fungi parasitici et saprophytici. Zeitschr Parasitenkunde 3: 260–516

Wollenweber HW and Reinking OA (1935) Die Fusarien. Ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parey, Berlin, Germany