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Genetic diversity and pathogenicity of *Fusarium* and *Neocosmospora* associated with cultivated and wild *Asparagus officinalis* in The Netherlands

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Summary. Fusarium crown and root rot is one of the main threats to asparagus production worldwide. The aetiology of the disease, attributed to several species of *Fusarium* and *Neocosmospora*, has not been re-evaluated in the context of recent advances in *Fusarium* taxonomy, and relevant studies in The Netherlands are scarce and outdated. *Asparagus officinalis* plants and associated soil samples were assessed from 38 cultivated fields and one location where this plant naturally occurs in The Netherlands. A total of 164 specimens were obtained yielding 551 fungal strains. Fungal identification according to DNA sequencing and phylogenetic analyses based on fragments of the translation elongation factor 1-alpha (*tef1*) and the DNA-directed RNA polymerase II second largest subunit (*rpb2*) showed that these isolates belonged to 20 taxa (*Fusarium acuminatum*, *F. annulatum*, *F. avenaceum*, *F. carminascens*, *F. celtidicola*, *F. clavus*, *F. cugenangense*, *F. culmorum*, *F. equiseti*, *F. flagelliforme*, *F. flocciferum*, *F. inflexum*, *F. nirenbergiae*, *F. odoratissimum*, *F. oxysporum*, *F. redolens*, *F. vanleeuwenii*, *Neocosmospora geoasparagicola*, *N. solani* and *N. stercicola*), of which 11 species (*Fusarium avenaceum*, *F. carminascens*, *F. celtidicola*, *F. clavus*, *F. cugenangense*, *F. flagelliforme*, *F. flocciferum*, *F. inflexum*, *F. odoratissimum*, *F. vanleeuwenii* and *N. stercicola*) are first reports on *A. officinalis*. These results, and testing of Koch's postulates on *A. officinallis* plantlets, showed that *F. annulatum*, *F. culmorum*, *F. redolens*, *F. nirenbergiae* and *N. solani* were the most prevalent and aggressive species. In contrast, although *F. oxysporum* is pathogenic to *A. officinalis*, it was among the least prevalent species encountered in this study.

Keywords. *Asparagaceae*, fungal diversity, fusarioid, pathogens, phylogeny.

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INTRODUCTION

Asparagus officinalis has historically been cultivated in The Netherlands, and at present remains an economically significant crop. Primarily grown as a cash crop in the southern-most provinces of Limburg and Noord-Brabant (CBS, 2025), wild plants also occur in sandy and loamy soil on the dunes of South and North Holland and Zeeland provinces. Dutch asparagus production has fluctuated between 13.3 to 17.5 million kg p. a. over the past 11 years, and at its lowest production in 2024 (CBS, 2025). Currently, both world market value and production of asparagus are increasing, and despite a decline in Dutch cultivation, most of the country's local asparagus production is still destined for external markets. Almost 60% of the total 14.5 million kg produced in 2023 was exported, valued at US$68.22 million (CBS, 2025; World Integrated Trade Solution, 2025).

Fusarium crown and root rot pose a significant threat to asparagus production, both in The Netherlands and elsewhere. The disease is caused by diverse species of *Fusarium* or related fusarioid taxa, and is characterized by vascular discolourations with typical red-brown elliptical lesions affecting asparagus root systems, and sometimes extending to the crowns. Asparagus plants can be infected through various routes, including root, root tip, or stem wounds, and insect transmission, such as by the asparagus miner (*Ophiomyia simplex*, *Agromyzidae*, *Diptera*) (Elmer, 2015), while seed contamination can occur during harvesting when seeds are washed with water contaminated with diseased host tissues (Block and Bollen, 1995). Plants can be affected at any growth stage, although disease symptoms are more commonly observed in established fields (Elmer *et al*., 1997; Elmer, 2015). The disease leads to stunted ferns with a gradual decline in productivity, and plants show progressive yellow discolourations, followed by necroses and stem death (Elmer, 2015; Aegerter *et al.,* 2016). Due to a lack of effective countermeasures, preventive measures are the only available option for disease management. However, finding pathogen-free substrates for asparagus cultivation is challenging due to the biology of the fungal pathogens, being able to persist in the soil for decades by producing highly resistant and resilient propagules (Block and Bollen, 1995). Wind-blown pathogen infested soil, rain splash, soil movement and contamination of tools and machinery can easily spread the disease and compromise neighbouring fields or glasshouses (Block and Bollen, 1995). Presence of the fungi in seeds and crowns can also spread the disease to distant localities (Corpas-Hervias *et al*., 2006; Elmer, 2015).

Fungal species traditionally associated with fusarium root rot of asparagus include *Fusarium acuminatum*, *F. annulatum*, *F. oxysporum* (*F. oxysporum* f. sp. *asparagi*), and *F. redolens* (*F. redolens* f. sp. *asparagi*), with *F. redolens* also commonly associated with spear rot, and *Neocosmospora solani* (*F. solani*); while *F. culmorum* often causes stem base and crown rot (Van Bakel and Krom-Kerstens, 1974; Baayen *et al*., 2000; Corpas-Hervias *et al*., 2006; Borrego-Benjumea *et al*., 2014; Elmer, 2015). All these species are soil-borne, cosmopolitan, and plant pathogenic, and are known to occur in The Netherlands. However, not all these species have been associated with asparagus disease in this country.

Recent advances in the taxonomy of *Fusarium* and related taxa, particularly in *F. oxysporum* and *Neocosmospora*, suggest that a larger number of pathogenic species could be involved as asparagus pathogens (Lombard *et al*., 2019; Sandoval-Denis *et al*., 2019; Crous *et al*., 2022). This is due to the known genotype diversity of relevant *formae speciales* (Baayen *et al*., 2000; Corpas-Hervias *et al*., 2006; Elmer, 2015).

This present study aimed to identify and report fusarioid fungal species associated with root-rot of cultivated and wild *A. officinalis* in The Netherlands, and to determine the ability of these fungi to cause disease under controlled laboratory conditions.

MATERIALS AND METHODS

Specimen collection

Asparagus plants and adjacent soil samples were collected from 38 asparagus fields in Limburg, The Netherlands. Wild asparagus plants, soils and rhizosphere samples were also collected from the Berkheide dune area, Hollandse Duinen National Park, Wassenaar, South Holland (Table 1). Additionally, 123 fusarioid isolates were obtained from working collections of two laboratories belonging to Bejo Zaden B.V. (Warmenhuizen, The Netherlands). These isolates were from numerous *Asparagus* spp. tissues, including 44 isolates originating from *Asparagus* spp. seeds (Supplementary Table S1). Plant material and soils were deposited in sterile plastic bags and maintained at 10 °C until processing.

Fungal isolations

Plant material was washed thoroughly under running tap water to remove any soil remnants. Different plant tissues (*i.e*., crowns, roots, or stems) were processed independently. Seeds and fragments (ca. 5 × 5

Table 1. Origins and characteristics of sampled *Asparagus officinalis* plants.

Plant	Location	Variety	Plant age (years)	Origin of the variety
1	Limburg, Kessel	Backlim	3	Netherlands, Limseeds B.V.
2	Limburg, Kessel	Backlim	3	Netherlands, Limseeds B.V.
3	Limburg, Kessel	Grolim	3	Netherlands, Limseeds B.V.
4	Limburg, Kessel	Grolim	3	Netherlands, Limseeds B.V.
5	Limburg, Kessel	Grolim	3	Netherlands, Limseeds B.V.
6	Limburg, Kessel	Canticus	3	Netherlands, Bejo Zaden B.V.
7	Limburg, Kessel	Cumulus	3	Netherlands, Bejo Zaden B.V.
8	Limburg, Kessel	Cygnus	3	Netherlands, Bejo Zaden B.V.
9	Limburg, Kessel	Gynlim	3	Netherlands, Limseeds B.V.
10	Limburg, Kessel	Inbred breeding cross	3	Netherlands, Bejo Zaden B.V.
11	Limburg, Kessel	Inbred breeding cross	3	Netherlands, Bejo Zaden B.V.
12	Limburg, Kessel	Inbred breeding cross	3	Netherlands, Bejo Zaden B.V.
13	Limburg, Kessel	Grolim	3	Netherlands, Limseeds B.V.
14	Limburg, Kessel	Guelph Millenium	3	Canada, University of Guelph
15	Limburg, Kessel	Herkolim	3	Netherlands, Limseeds B.V.
16	Limburg, Kessel	Inbred breeding cross	3	Netherlands, Bejo Zaden B.V.
17	Limburg, Kessel	Inbred breeding cross	3	Netherlands, Bejo Zaden B.V.
18	Limburg, Kessel	Inbred breeding cross	2	Netherlands, Bejo Zaden B.V.
19	Limburg, Kessel	Prius	2	Netherlands, Bejo Zaden B.V.
20	Limburg, Kessel	Roem van Brunswijk	2	Germany
21	Limburg, Kessel	Schneekopf	2	Germany
22	Limburg, Kessel	Inbred breeding cross	2	Netherlands, Bejo Zaden B.V.
23	Limburg, Kessel	Interspecific cross	1	Netherlands, Bejo Zaden B.V.
24	Limburg, Kessel	Interspecific cross	1	Netherlands, Bejo Zaden B.V.
25	Limburg, Kessel	Interspecific cross	1	Netherlands, Bejo Zaden B.V.
26	Limburg, Kessel	Interspecific cross	1	Netherlands, Bejo Zaden B.V.
27	Limburg, Kessel	Cygnus	Unknown	Netherlands, Bejo Zaden B.V.
28	Limburg, Kessel	Cygnus	Unknown	Netherlands, Bejo Zaden B.V.
29	Limburg, Kessel	Inbred breeding cross	2	Netherlands, Bejo Zaden B.V.
30	South Holland, Berkheide	Wild	Unknown	Not applicable
31	South Holland, Berkheide	Wild	Unknown	Not applicable
32	South Holland, Berkheide	Wild	Unknown	Not applicable
33	South Holland, Berkheide	Wild	Unknown	Not applicable
34	Limburg, Kessel	Prius	8	Netherlands, Bejo Zaden B.V.
35	Limburg, Kessel	Bacchus	8	Netherlands, Bejo Zaden B.V.
36	Limburg, Kessel	Gynlim	8	Netherlands, Bejo Zaden B.V.
37	Limburg, Kessel	Backlim	8	Netherlands, Bejo Zaden B.V.
38	Limburg, Kessel	Grolim	8	Netherlands, Bejo Zaden B.V.
39	Limburg, Kessel	Cumulus	8	Netherlands, Bejo Zaden B.V.
40	Limburg, Kessel	Erasmus	8	Netherlands, Bejo Zaden B.V.
41	Limburg, Kessel	Cygnus	8	Netherlands, Bejo Zaden B.V.

mm) of symptomatic and asymptomatic tissues were excised, surface-sterilized by immersion in 10% sodium hypochlorite solution for 1 min, followed by immersion in 70% ethanol for 30 s, and rinsing three times in sterilised distilled water. Tissue fragments were then dried using sterilised paper towels, and up to five fragments were aseptically distributed on medium surfaces of 9 cm diam. Petri dishes containing different culture media, including: Malt Extract agar (MEA; Crous *et al.*, 2019), MEA amended with 2% chloramphenicol (MEA + CAF), Synthetic Nutrient Poor agar (SNA; Nirenberg, 1976), and Pentachloronitrobenzene agar (PCNB; Crous *et al.*, 2021). Plates were incubated at room temperature (approx. 24 °C) in darkness, checked daily for fungal

growth, and single conidium cultures were obtained by transferring individual germinating conidia onto Oatmeal agar plates (OA; Crous *et al*., 2019) with the aid of low magnification microscopy. Soil and rhizosphere samples were processed with a modification of the protocol of Groenewald *et al.* (2018). Samples were aseptically sieved to remove any plant remains and other large debris, and were deposited in open 9 cm diam. Petri dishes and were then left to dry under a laminar flow cabinet. One g of dry soil was then serially diluted from 10^{-2} to 10^{-4} in water containing 0.5 g L^{-1} agarose, and 1 mL of each dilution was spread over the surface of solid MEA + CAF or PCNB agar in Petri plates. The plates were then incubated and single conidium isolations were carried out as described above.

Fungal identifications

All isolates were subjected to total DNA extraction, using the Wizard® Genomic DNA Purification Kit (Promega), following the manufacturer's instructions, from mycelium scraped from colonies grown on MEA for 3 to 5 d at 24 °C in darkness. Fragments of two genomic regions, the translation elongation factor 1-alpha (*tef1*) and the DNA-directed RNA polymerase II second largest subunit (*rpb2*), were then PCR amplified using the primer pairs EF-1/EF-2 (O'Donnell *et al*., 1998) and RPB2-5f2/fRPB2-7cr (Liu *et al*., 1999), using the PCR protocols according to Crous *et al.* (2021). Amplicons were sequenced in both directions using the primers indicated above on a Hitachi 3730xl DNA analyser (Applied Biosystems Inc.). Consensus sequences were assembled from forward and reverse sequences using Geneious Prime v. 2023.2.1 (Biomatters Inc.). Sequences generated in this study were deposited in GenBank (Supplementary Table S1).

Sequences from each locus newly generated in the present study, as well as type and reference sequence data retrieved from Fusarioid-ID (www.fusarium.org) and GenBank (Supplementary Table S2), were aligned using MAFFT v. 7 (Katoh and Standley, 2013) Multiple Sequence Alignment (MSA) as provided by the EMBL's European Bioinformatics Institute website (www.ebi.ac.uk/jdispatcher/msa/mafft), were exported and manually corrected in MEGA v. 6.06 (Tamura *et al*., 2013), and individual loci datasets were combined using SequenceMatrix v. 1.9 (Vaidya *et al*., 2011).

Phylogenetic analyses were carried out using Maximum-Likelihood (ML) and Bayesian (BI) algorithms. For ML, RAxML-HPC2 was used on XSEDE v. 8.2.12 (Stamatakis, 2006) as implemented in the CIPRES Science Gateway (Miller *et al*., 2010), and additional calculations were made using IQ-TREE multicore v. 2.1.2 COVID-edition (Minh *et al*., 2020). For RAxML runs, the default best-fit model (General Time Reversible) and parameters were retained, and branch support was calculated using 1,000 non-parametric bootstrap inferences. Evolutionary models for IQ-TREE were calculated for each partition according to the TESTNEW option of ModelFinder (Kalyaanamoorthy *et al*., 2017) as implemented in IQ-TREE, and branch support was estimated with 1,000 replicates of ultrafast bootstrap approximation (UFBoot; Hoang *et al*., 2018). For BI, evolutionary models were calculated for each gene partition in MrModelTest v. 2.3 (Posada and Crandall, 1998; Nylander, 2004), selected according to the Akaike information criterion. The BI analyses were set up on MrBayes v. 3.2.7a (Ronquist *et al*., 2012) as implemented in the CIPRES Science Gateway, and included 5 M generations with the stop-rule option on, including four incrementally heated MCMC chains. Trees were sampled every 1,000 generations and runs were checked for convergence by average standard deviation of split frequencies below 0.01. Consensus trees (50%) and posterior probabilities (PP) were calculated after discarding 25% of the initial samples as the burn-in fraction. Alignments, phylogenetic trees and supplementary data were deposited in www.figshare.com (doi: https://doi.org/10.6084/m9.figshare.33269964).

Pathogenicity testing

To test Koch's Postulates, an *in vitro* method was developed by adapting and optimizing the protocols of Blok and Bollen (1995), Wong and Jeffries (2006), Corpas-Hervias *et al.* (2006) and Borrego-Benjumea *et al.* (2014). Seeds of *Asparagus officinalis* "Cumulus F1" (Bejo Zaden B.V.) were surface sterilized by immersion in a solution of 5% sodium hypochlorite and 0.05% Tween 20 for 1 min under constant agitation, followed by five rinses in abundant sterilized distilled water. The sterilized seeds were then aseptically placed in moisture chambers, and incubated at 27 °C in darkness, and were checked daily for 7 to 8 d until a 2 to 5 mm long radicle from each seed was visible. Once germinated, the seeds were placed onto 2.2 g L^{-1} Murashige and Skoog agar (MS; Sigma-Aldrich) amended with 5 g L^{-1} sucrose, with 8 mL of MS per glass test tube (18 mm diam.). Plantlets were then incubated for 1 to 2 weeks under a day/night cycle (23 °C for 16 h under cool fluorescent light, followed by 18 °C for 8 h in darkness) until root systems and 3 to 4 cm long shoots had developed. These plants were then inoculated by transferring an agar plug (*ca.* 5 × 5 mm from an OA plate containing a 7 to 14-d-old, well-spor-

ulated fungal culture, incubated at 24 °C under constant near UV light (nUV), located on the agar surface), near the base of the roots. Quantification experiments showed that this inoculum was equivalent to 1×10^5 conidia mL^{-1}. Challenged plants were then incubated for up to 21 d in a day/light cycle (as indicated above). Five replicates (plants) were used for each fungal isolate. Control plants were inoculated with sterile OA plugs. Presence/absence and extent of root lesions were observed daily, and pathogenicity was scored according to observed symptom severity as: 0 (absence of symptoms), 1 = browning of roots without evident lesions; 2 = presence of few (< 10) discernible lesions on primary roots, with restricted secondary roots; 3 = abundant (> 10) lesions on primary roots, with severely restricted secondary roots; and 4 = plant death. Severity scores were used to calculate the disease incidence (I = [N affected plants/N plants examined] × 100) and disease severity index (DSI = [sum of numerical ratings/(N examined plants × maximum possible grade)] × 100). To exclude duplicate isolates from the pathogenicity experiments and to avoid data repetition, a selection of 487 unique fusarioid isolates was carried out by comparing the isolate origin and *tef1* haplotypes of all strains where this information was available.

Pathogenicity results were statistically analysed using one-way ANOVA, followed by Student´s t-test assuming equal variances, with Bonferroni´s correction for multiple comparisons, using the data analysis module in MS Excel v. 2019. Adjusted *P* values (P_{adj}) are shown after each comparison in Results (below). Graphs were made using the same software and coloured using Adobe Illustrator v. 30.0.

RESULTS

Samples and fungal isolation

Plant material and soil samples were obtained from 38 cultivated fields in Limburg, plus one location where wild *A. officinalis* naturally occurs (Table 1). A total of 41 plants were dug and processed, corresponding to 13 different commercial asparagus varieties (Bacchus, Backlim, Canticus, Cumulus, Cygnus, Erasmus, Grolim, Guelph Millenium, Gynlim, Herkolim, Prius, Roem van Brunswijk, and Schneekopf), seven breeding crosses, four interspecific crosses, and wild *A. officinalis* plants. The age of the sampled plants varied from 1- to 8-years-old for cultivated fields while for wild asparagus it was undetermined. A total of 551 fungal isolates were obtained belonging to the fusarioid genera *Fusarium* or *Neocosmospora*. Exclusion of identical isolates from one origin based on *tef1* haplotypes resulted in 487 unique isolates.

Fusarioid fungi were recovered from every type of sample studied. Most fusarioid isolates were obtained from asparagus tissues (380; 68.9%), including: from roots (243 isolates; 44%), crowns (55; 10%), seeds (44; 8%), seedlings (32; 6%), or from stems (six; 1%). Soil (112 isolates; 20.3%) and rhizosphere (59 isolates; 10.7%) accounted for a third of the total number of fungal strains (Table 2).

Twenty species were identified based on molecular data, 17 of which belonged to seven *Fusarium* species complexes (SC), namely: *F. celtidicola* in the *Fusarium celtidicola* SC (FCCSC); *F. annulatum* in the *F. fujikuroi* SC (FFSC); *F. clavus*, *F. equiseti* and *F. flagelliforme* in the *F. incarnatum-equiseti* SC (FIESC); *F. carminascens*, *F. cugenangense*, *F. inflexum*, *F. nirenbergiae*, *F. odoratissimum*, *F. oxysporum* and *F. vanleeuwenii* in the *F. oxysporum* SC (FOSC); *F. redolens* in the *F. redolens* SC (FRSC), *F. culmorum* in the *F. sambucinum* SC (FSAMSC), *F. acuminatum*, *F. avenaceum* and *F. flocciferum* in the *F. tricinctum* SC (FTSC); and three species in the genus *Neocosmospora* (formerly the *F. solani* SC; *N. geoasparagicola*, *N. solani* and *N. stercicola*) (Figure 1, Table 2). The most prevalent species was *Fusarium nirenbergiae* (34%), which was also the only species found in all the sample types, and was the most prevalent species from every origin except from seeds or soil. The second most prevalent species was *Fusarium redolens* (23%), being the predominant species in soil and the second most common fusarioid species in roots. This fungus was recovered with less frequency from rhizospheres, crown tissues, and seedlings, but was not recovered from seeds or stems. The third most prevalent species, *Fusarium annulatum* (15%), was the most common fusarioid fungus isolated from seeds (95% of the total isolations from seeds), but was also found in every other sample type except from stems. This species was one of the most common isolates found in plant tissues and soils from cultivated asparagus in this study, but was not found associated with asparagus in the wild environment. The fourth and fifth most isolated species were *Neocosmospora solani* and *Fusarium culmorum*, comprising, respectively, 7.6% and 4% of the total isolations. These two fungi were mostly isolated from roots and soil (*N. solani*), and seedlings (*F. culmorum*).

Less commonly isolated species included: *F. carminascens* (19 isolates), *N. stercicola* (16 isolates), *F. avenaceum* (10 isolates), *F. vanleeuwenii* (eight isolates), *N. geoasparagicola* (seven isolates), *F. acuminatum* and *F. flagelliforme* (six isolates each), *F. inflexum* (three isolates), *F. clavus*, *F. odoratissimum* and *F. oxysporum* (two isolates each), and *F. cugenangense, F. equiseti*, *F. celtidicola* and *F. flocciferum* (one isolate each) (Table 2).

Table 2. Origins of fusarioid fungal isolates, according to asparagus plant or soil sample type.

Fungus name	Origin (number of isolates)							
	Crown	Rhizosphere	Root	Seed	Seedling	Soil	Stem	Total
Fusarium acuminatum	2				4			6
Fusarium annulatum	10	10	20	42	1	3		86
Fusarium avenaceum	1	2	4		3			10
Fusarium carminascens		3	9			7		19
Fusarium celtidicola							1	1
Fusarium clavus						2		2
Fusarium cugenangense		1	0					1
Fusarium culmorum	3	2	2		10	3	1	21
Fusarium equiseti						1		1
Fusarium flagelliforme			1		3	2		6
Fusarium flocciferum						1		1
Fusarium inflexum			3					3
Fusarium nirenbergiae	27	22	97	1	10	28	4	189
Fusarium odoratissimum			2					2
Fusarium oxysporum	1		1					2
Fusarium redolens	5	11	78		1	33		128
Fusarium vanleeuwenii	1		4	1		2		8
Neocosmospora geoasparagicola						7		7
Neocosmospora solani	2	7	17			16		42
Neocosmospora stercicola	3	1	5			7		16
Total	55	59	243	44	32	112	6	551

Pathogenicity testing

In vitro pathogenicity testing of 487 unique isolates showed that a majority (408; 84%) could induce lesions on asparagus seedling roots and crowns. Disease incidence (% of affected replicates) ranged from 20 to 100%, with 43% (209 isolates) inducing lesions in all five plant replicates. The degree of root damage was, in general, highly variable, with most challenges resulting in either absence of root lesions (severity score 0; 35% of isolates) or great compromise of the entire root system resulting in plant death (severity score 4; 33.3% of isolates). Intermediate severity scores (1 to 3) were observed in, respectively, 6, 12.8 or 12.6% of the total assays (Figures 2 and 3).

Among the most prevalent fungal species in the dataset, 99% of isolates of *F. annulatum*, 95% of *F. culmorum*, 92% of *F. redolens*, 89% of *F. nirenbergiae*, and 67% of *N. solani* induced symptoms in asparagus plantlets, affecting either root or crown tissue alone, or as combinations of both. According to calculated I and DSI indices, the two most pathogenic and aggressive species were *F. culmorum* (I = 78%; DSI = 64%) and *F. annulatum* (I = 67%; DSI = 55%) (Figure 2). Seventy-seven percent of all *in vitro* asparagus inoculations with *F. culmorum*, and 37% of inoculations with *F. annulatum*, led to plant death at the end of the trial, in both cases mostly due to effects on the host plant crowns, with minimal presence or absence of root lesions. No statistically significant differences in pathogenicity were observed between these two species. However, both species were associated to significantly more aggressive disease *in vitro* than most of the common asparagus pathogens tested here, including *F. redolens*. This species (I = 74%; DSI = 44%) differed significantly from *F. annulatum* in both incidence (P_{adj} = 0.01) and disease severity (P_{adj} = 1.89×10^{-8}). Incidence of *F. redolens* was comparable to that of *F. culmorum* (P_{adj} = 0.59), although disease severity was significantly less (P_{adj} = 3.03×10^{-7}). *Fusarium nirenbergiae* (I = 69%; DSI = 44%) did not differ from *F. redolens* in either parameter, and showed greater incidence than *F. annulatum* (P_{adj} = 1.59×10^{-4}), but not than *F. culmorum* (P_{adj} = 0.26). Disease severity was significantly lower in *F. nirenbergiae* than that from *F. redolens* (P_{adj} = 6.72×10^{-9}) or from *F. annulatum* (P_{adj} = 6.96×10^{-7}). *Neocosmospora solani* caused the least disease incidence and severity (I = 28%; DSI = 18%), differing significantly from all the other fungi mentioned above in both parameters ($P_{adj} \leq 3.86 \times 10^{-5}$ for incidence, $\leq 1.22 \times 10^{-21}$ for disease severity). Less represented species induced varied levels of disease, among which *F. oxyspo-*

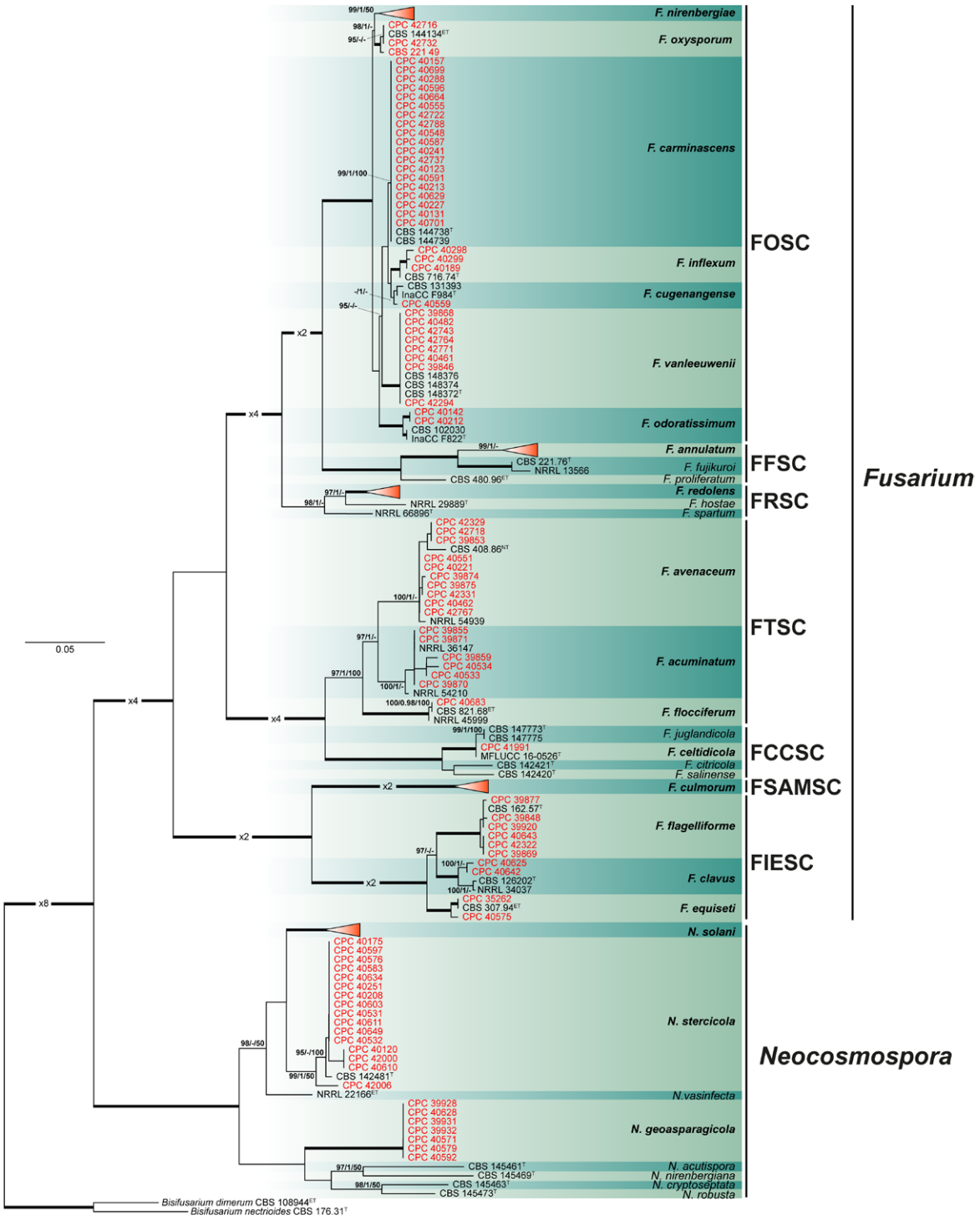

Figure 1. Maximum Likelihood (IQ-TREE) phylogram inferred from combined *rpb2* and *tef1* datasets of representative isolates of *Fusarium* and *Neocosmospora*, plus 551 fungal isolates obtained from asparagus tissues and soils. Numbers at nodes indicate support values (IQ-TREE-BS / BI-PP / RAxML-BS) greater than 70 % (BS) and 0.95 (PP). Thickened branches indicate full support (BS = 100, PP = 1). The tree is rooted to *Bisifusarium dimerum* CBS 108944 and *B. nectrioides* CBS 176.31. Due to the abundance of strains, clades representing *F. annulatum*, *F. culmorum*, *F. nirenbergiae*, *F. redolens* and *N. solani* were collapsed to facilitate display. Culture collection abbreviations are listed in Supplementary Table S2. Ex-epitype, ex-neotype or ex-type strains are indicated by, respectively, ET, NT or T. *Fusarium* species complexes (SC) are abbreviated as: FCCSC = *F. celtidicola* SC, FFSC = *F. fujikuroi* SC, FIESC = *F. incarnatum-equiseti* SC, FOSC = *F. oxysporum* SC, FRSC = *F. redolens* SC, FSAMSC = *F. sambucinum* SC, and FTSC = *F. tricinctum* SC.

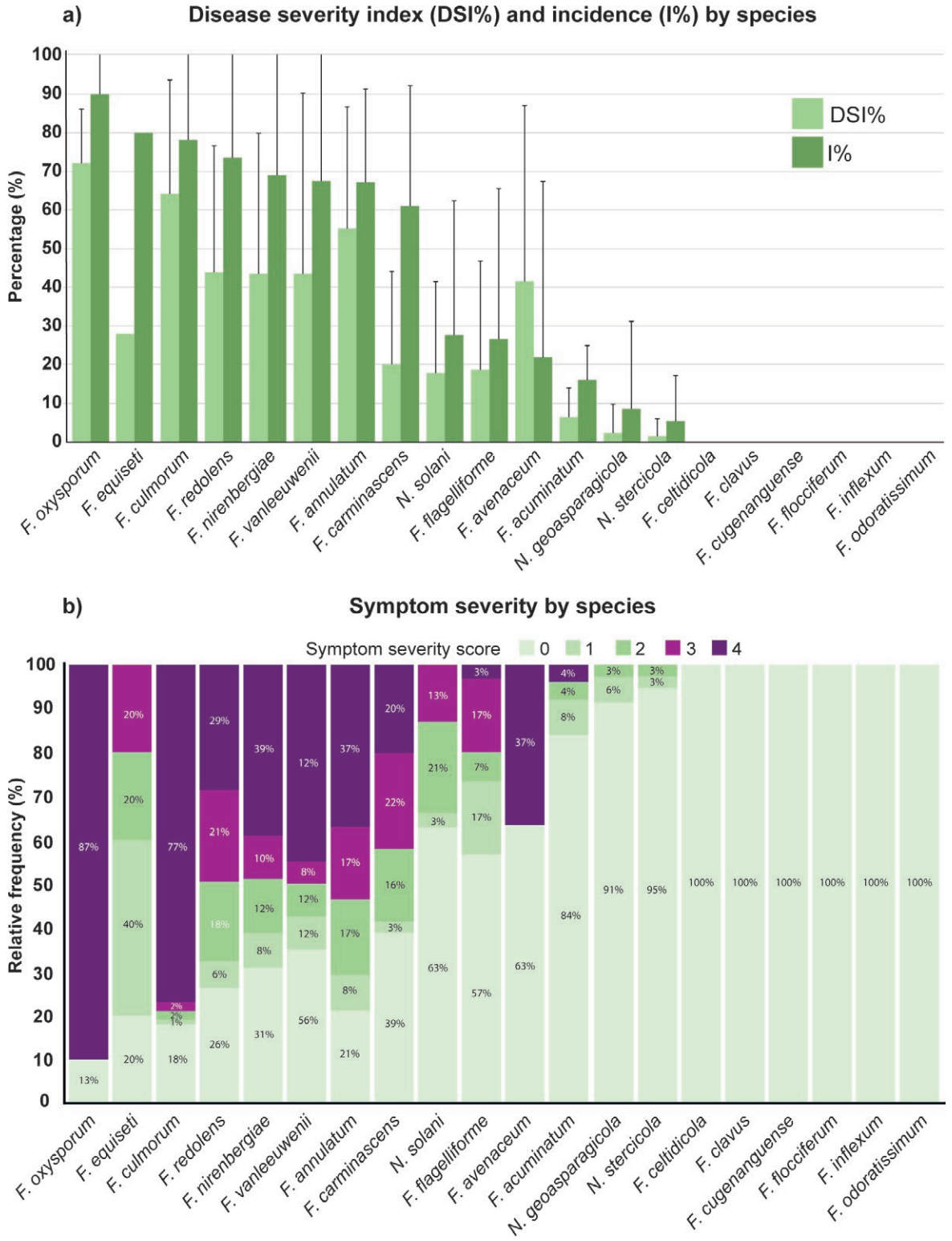

Figure 2. a: Calculated mean Disease Severity Indices (DSI%) and incidences (I%) per species, for fungal taxa ordered according to their indices, from high (left) to low (right). Lines above bars indicate standard deviations. b: Observed symptom severity scores per species, as: 0 = no symptoms, 1 = browning of roots, without evident lesions; 2 = few discernible lesions on primary roots, with restricted secondary roots; 3 = abundant lesions on primary roots, with severely restricted secondary roots; 4 = plant death. Fungal species are ordered alphabetically from left to right.

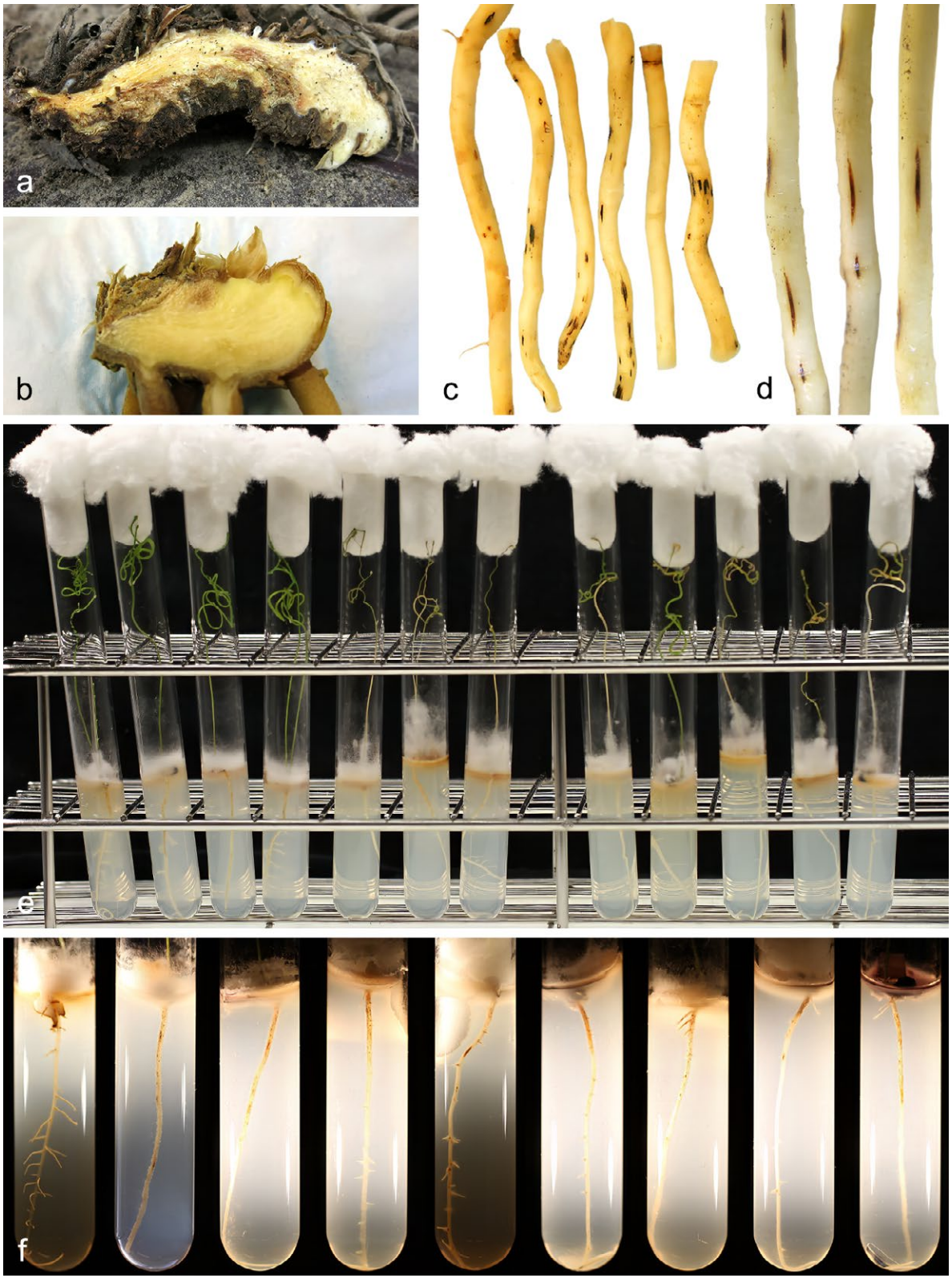

Figure 3. Asparagus lesions attributed to fusarioid species. a, b: naturally occurring crown lesions on cultivated *Asparagus officinalis*. c, d: typical root lesions on a cultivated plant (c) and a wild plant (d). e: experimental setup showing diverse symptoms on infected *A. officinalis* plantlets. f: detail of different degrees of root symptoms on infected *A. officinalis* plantlets.

rum and other members of the FOSC (*i.e.*, *F. carminascens*, *F. vanleeuwenii, F. cugenangense*), and *F. equiseti* from the *Fusarium incarnatum-equiseti* species complex (FIESC) caused high incidences (I = 56 to 100%). Despite its low isolation frequency (two isolates), *Fusarium oxysporum* also showed high aggressiveness (DSI = 72%, and causing 87% plant death with extensive crown symptoms, but without root lesions). However, these observations are not statistically supported, due to the limited number of isolates in this dataset. *Fusarium avenaceum* (belonging to the *Fusarium tricinctum* species complex, FTSC) gave relatively low disease incidence (I = 22%), and did not attack the asparagus root systems. This fungus, however, was an aggressive crown pathogen (DSI = 42%), with 37% of inoculations leading to plant death. Isolates of *F. acuminatum*, *N. stercicola* and *N. geoasparagicola* were only associated with mild root and crown symptoms (I ≤ 16%; DSI ≤ 6%). None of the *F. celtidicola*, *F. clavus*, *F. flocciferum*, *F. inflexum*, or *F. odoratissimum* isolates induced symptoms on inoculated asparagus seedlings.

A subset of 37 isolates (*N. solani* n = 13, *N. stercicola* n = 8, *F. redolens* n = 4, *F. carminascens*, *F. flagelliforme* and *F. nirenbergiae* each n = 3 each, *F. annulatum* n = 4, and *F. culmorum* n = 1) was associated with enhanced asparagus growth in the bioassays, producing taller, thicker and greener stalks than the inoculation controls. This phenotype was constant between repetitions and had no apparent association with fungal species or strain origin (see isolate details in Table S1).

DISCUSSION

Twenty species of *Fusarium* and *Neocosmospora* were isolated from *Asparagus officinalis* tissues and associated soils, and were identified by using multilocus phylogenetic analysis, and their pathogenicity was assessed using an *in vitro* system. The present study is the most extensive in terms of fusarioid diversity to have been carried out on asparagus.

Fusarium nirenbergiae was the most frequently encountered species in this dataset, and until recently was an unnamed phylogenetic lineage, hidden within a too comprehensive concept of *F. oxysporum sensu lato* (*s. l.*) (Snyder and Hansen 1940). After its formal description (Lombard *et al.* 2019), *F. nirenbergiae* has emerged as a ubiquitous species in the FOSC (Crous *et al.* 2021, Wang *et al.* 2022, Han *et al.* 2023). Nevertheless, its host range and pathogenicity, including against *A. officinalis*, remains to be determined and contrasted against historical data for *F. oxysporum s. l.* Previous studies on the prevalence of fusarioid asparagus pathogens in The Netherlands date from the 1990s, and were based on old taxonomic concepts (Blok and Bollen, 1995; Baayen *et al.*, 2000). Similarly, modern studies from asparagus producing countries, although based on molecular data, have often relied on outdated *Fusarium* taxonomy. This is of particular relevance when assessing the most prevalent asparagus pathogens, most of which have recently undergone important taxonomic changes (Lombard *et al.*, 2015, 2019; Sandoval-Denis *et al.*, 2019; Crous *et al.*, 2021). Nevertheless, as observed here, the distribution of the most common species is, in general, congruent with previous reports in The Netherlands and elsewhere (Block and Bollen, 1995; Elmer *et al.*, 1997; Wong and Jeffries, 2006; Farr *et al.*, 2023; Buitrago-Acosta *et al.*, 2025). However, and contrary to previous indications, *F. oxysporum* was not the most commonly associated species on cultivated asparagus and was found on one crown and one root sample in the present study.

Previous knowledge about asparagus-related *F. oxysporum* is, without exception, based on old species concepts. The name *F. oxysporum s. l.* has traditionally and erroneously been employed as a wild card to classify a genetically diverse group of different species with very similar phenotypes (O'Donnell *et al.*, 2004; Lombard *et al.*, 2019; McTaggart *et al.*, 2021), therefore perpetuating the incorrect application of the name *F. oxysporum*. Conversely, the widespread availability of sequence data has recently accelerated species descriptions within FOSC, occasionally resulting in the recognition of poorly delimited or taxonomically redundant taxa that complicate identification and the interpretation of phylogenetic analyses (Lombard *et al.*, 2019; Tan and Conroy, 2024; Tan and Shivas, 2024). Such taxa were therefore not adopted in the identification of isolates in the present study. However, isolates potentially assignable to these entities and their phylogenetic relationships are documented in the extended phylogenetic analysis available in FigShare (doi:10.6084/m9.figshare.33269964). The alternative classification of this group of pathogens according to the f. sp. nomenclature (*e.g.*, *F. oxysporum* f. sp. *asparagi*, Cohen and Heald (1941)) has resulted in a similar problem since they do not cluster in monophyletic groups, when compared to formal taxonomic ranks, grouping different entities here demonstrated to be different species. Most of these species have only recently been formally described, and were shown in the present study to induce asparagus disease (Lombard *et al.*, 2019). As demonstrated here, the traditional concepts of *F. oxysporum s. l.* and *F. oxysporum* f. sp. *asparagi* encompass a series of distinct species (*F. nirenbergiae*, *F. carminascens*, *F. cugenangense*, *F. vanleeuwenii*, *F. inflexum*, *F. odoratissimum*, and *F. oxyspo-*

rum sensu stricto [*s. st.*]), all belonging to the FOSC and virtually indistinguishable morphologically. The relevance of the reassessment of *Fusarium* taxonomy is highlighted by the markedly different levels of pathogenicity of these fungi towards *A. officinalis*.

Fusarium redolens, the second most prevalent species in the present study, was for a long time treated either as a synonym or a variety of *F. oxysporum* (Snyder and Hansen, 1940; Gordon, 1952; Bilai, 1955; Block and Bollen, 1995; Baayen *et al*., 2000). This species is a recognized asparagus pathogen commonly isolated from fields in Western and Northern Europe, including The Netherlands (Baayen *et al*., 2000; Schroers *et al*., 2004; Wong and Jeffries, 2006), and North America (Vujanovic *et al*., 2006; Borrego-Benjumea *et al*., 2014). Similarly, the third most isolated species in this series, *F. annulatum*, is also a documented asparagus pathogen with broad distribution that includes Argentina (Lori *et al*., 1998), Australia and New Zealand (Elmer *et al*., 1997), Canada, and the United States of America (Elmer, 1990; Baayen *et al*., 2000; Vujanovic *et al*., 2006; Borrego-Benjumea *et al*., 2014), Japan (Nahiyan *et al*., 2011), The Netherlands (Baayen *et al*., 2000), South Africa (Schreuder *et al*., 1995), Spain (Wong and Jeffries, 2006) and the United Kingdom (Wong and Jeffries, 2006).

Fusarium annulatum has often been erroneously reported as *F. proliferatum*, due to the confusing taxonomic history of these two species. These two taxa, both segregates of *F. moniliforme s. l.* (Seifert *et al*., 2003) and previously considered synonyms, are now confined to two genealogically exclusive clades, of which the one representing the widely distributed and well-recognised plant pathogenic species is now assigned to *F. annulatum* (Yilmaz *et al*., 2021). Despite being a cosmopolitan species, only one report has mentioned isolation of *F. annulatum* (as *F. proliferatum*) from asparagus fields in The Netherlands (Baayen *et al*., 2000;), with no clear indication of its origin. The apparent absence of this species from Dutch soils was probably due to low soil temperatures, because this species is more commonly isolated in warm regions, and does not survive well in soils without plant hosts (Block and Bollen, 1996). This species was one of the most commonly found in the present study in plant tissues and soils from cultivated asparagus, but was not found associated with asparagus in the wild environments, suggesting introduction and spread through agricultural practices, or indicating a level of environmental adaptation.

The fourth most isolated species, *N. solani*, is commonly associated with soil-borne root diseases of several hosts (Sandoval-Denis *et al*., 2019), including *A. officinalis*, with reports from Brazil (Mendes *et al*., 1998), Europe, including the United Kingdom, The Netherlands, and Spain (Block and Bollen, 1996; Corpas-Hervias *et al*., 2006; Wong and Jeffries, 2006; Brizuela *et al*., 2020), Malaysia (Chehri *et al*., 2014), Canada and the United States of America (Damicone and Manning, 1985; Borrego-Benjumea *et al*., 2014), and South Africa (Schreuder *et al*., 1995). However, this species was non-to weakly aggressive to asparagus in the present study. *Neocosmospora* (previously the *Fusarium solani* SC) is another example of radical taxonomic change, particularly the segregation of this group to a separate genus based on molecular and morphological evidence (Lombard *et al*., 2015; Sandoval-Denis *et al*., 2019), and the epitypification and confinement of its historically most notorious species *N. solani* to a discrete phylogenetic lineage (Schroers *et al*., 2016). The name *N. solani* is no longer applicable to cover multiple phylogenetic entities (Snyder and Hansen, 1941).

Contrary to all the species discussed above, there is a broad taxonomic consensus regarding *F. culmorum*, the fifth most commonly isolated species in the present study. This species is a notable asparagus pathogen with wide distribution including Brazil (Mendes *et al*., 1998), China (Tai, 1979; Zhuang, 2005), Europe, including Italy (Richardson, 1990), The Netherlands (Block and Bollen, 1996), Spain (Brizuela *et al*., 2020) and the United Kingdom (Wong and Jeffries, 2006); and New Zealand (Pennycook, 1989).

While the above-mentioned species were approx. 85% of the total isolations in the present study, the remaining 15% of isolations were a heterogeneous assemblage of species with reduced isolation frequencies, from which only three taxa (*F. acuminatum*, *F. equiseti*, and *F. oxysporum s. st.*) are previously known from *Asparagus* spp. (Elmer *et al*., 1997; Vujanovic *et al*., 2006; Brizuela *et al*., 2020). The remaining 12 species (*i.e*., *F. avenaceum*, *F. carminascens*, *F. celtidicola*, *F. clavus*, *F. cugenangense*, *F. flagelliforme*, *F. flocciferum*, *F. inflexum*, *F. odoratissimum*, *F. vanleeuwenii*, *N. geoasparagicola*, and *N. stercicola*) are here reported for the first time in association with cultivated *A. officinalis*. In addition, a species novel to science, *N. geoasparagicola*, was discovered during this study, and has since been published elsewhere (Crous *et al*., 2022). This species was found only in soil samples from five cultivated fields; associated with five asparagus cultivars (Cygnus, Grolim, Guelph-Millenium, Schneekopf, plus an unidentified cultivar). It was recovered from asparagus-associated soils in two independent occasions separated by almost 2 years, and has since also been isolated from other types of soil in Limburg and Noord-Brabant (ARISE project, Westerdijk Fungal Biodiversity Institute, personal communication).

Verifications of Koch's postulates in this study largely agree with previous reports. However, the pathogenicity assays conducted in this study were designed as an initial screening, to identify potentially pathogenic *Fusarium* species under strictly controlled conditions. Consequently, the present results should be interpreted as evidence of pathogenic potential, while further validation under more representative cultivation conditions (mature asparagus plants under greenhouse or field conditions) remains warranted. Apart from slight regional evidence variations, pathogenicity data tend to allocate *Fusarium* species as the most relevant in terms of pathogenicity and virulence against asparagus (Elmer *et al*., 1997; Wong and Jeffries, 2006; Nahiyan *et al*., 2011; Borrego-Benjumea *et al*., 2014; Brizuela *et al*., 2020). However, as previously noted by Blok and Bollen (1995), the present study experimental set up could have overestimated the virulence of *F. culmorum*. Though in this dataset *F. culmorum* was isolated from every type of sample except asparagus seeds, unlike other species tested here, *F. culmorum* has been typically associated with stem base lesions leading to plant death (Van Bakel and Krom-Kerstens, 1974; Blok and Bollen, 1995). Due to the thin stem bases of young asparagus plants, and the inoculation method employed, these plant tissues are easy targets for this pathogen, which can quickly kill plantlets.

This is the first report of *F. avenaceum* from *A. officinalis*, However, this fungus has been reported in association with *Puccinia ranulipes* on *Asparagus laricinus* in South Africa (Doidge, 1950; Farr *et al*., 2023). Here this species was shown to induce moderate root symptoms on asparagus seedlings, yet when plant crowns were impacted, it can lead to plant death *in vitro*. Nevertheless, its behaviour under field conditions remains to be tested. Previous reports have shown moderate aggressiveness of *F. acuminatum* (Borrego-Benjumea *et al*., 2014) or no pathogenicity (Brizuela *et al*., 2020) against asparagus *in vitro*, which coincides with the present study observations. However, this species is a known plant root pathogen, and has a host range including *Asparagaceae* (*Ophiopogon japonicus*) (Tang *et al*., 2021). Conversely, none of the isolates of *F. celtidicola*, *F. clavus*, *F. flocciferum*, *F. inflexum*, or *F. odoratissimum* induced symptoms on inoculated asparagus. While none of the latter species have been previously reported from *Asparagus* spp., and are probably non-pathogenic, *F. flocciferum*, *F. inflexum* and *F. odoratissimum* are well-known pathogens of several plant species, particularly in *Fabaceae*, *Musaceae* and *Poaceae* (Farr *et al*., 2023).

Thirty-seven isolates belonging to seven *Fusarium* species (*i.e*., *F. redolens* [four isolates]), *F. carminascens*, *F. flagelliforme* and *F. nirenbergiae* [three isolates each], *F. annulatum* [two isolates]; and *F. culmorum* [one isolate], and particularly to two *Neocosmospora* species (*i.e*., *N. solani* [13 isolates], and *N. stercicola* [eight isolates]) were associated with improved plant growth in the present study bioassays. These isolates induced much taller, thicker and greener asparagus stalks compared to the controls, irrespective of the presence of root lesions on infected plants, which was a paradoxical phenomenon that seemed to not respond to any particular fungal species or isolate origin (data not shown). These experiments were not designed to test for beneficial effects of fungi, so further research will be required to confirm this effect, determine its magnitude, and consider underlying mechanisms.

CONCLUSIONS

The aetiology of asparagus fusarium disease is more complex than previously thought, with several species reported here as potential pathogens. Some of these species, despite being internationally ubiquitous, and known from several host species, are here reported for the first time from asparagus. Given the abilities of these fungi to induce asparagus disease *in vitro*, their importance for international asparagus global industries needs to be studied further. Several taxa recovered from lesions can also be isolated from healthy (or apparently healthy) asparagus plants, and several isolates obtained from healthy plants were able to induce symptoms in the *in vitro* assays. This demonstrates the plasticity and variety of adaptations known for fusarioid species (Ulaszewski *et al*., 2025), the complexity of host-fungal interactions, and the role of stochastic processes in the occurrence of disease. Furthermore, the presentation of asparagus disease is also known to vary in response to abiotic factors, which were not assessed here, while *in vitro* pathogenicity testing can be highly influenced by the age of the tested host plants (Elmer, 2015; Brizuela *et al*., 2020).

In terms of fungal diversity and despite limited sampling, this study has identified the greatest number of species isolated and tested for pathogenicity against *A. officinalis*. This diversity reflects improved species identification due to refined *Fusarium* taxonomy (Crous *et al*., 2021), and explains the differences in pathogenicity observed here with respect to traditionally encountered taxa (*F. oxysporum s. l.*). Re-evaluations of pathogen distribution and virulence, according to modern techniques and updated taxonomic concepts and classifications, will lead to increased understanding of species biology, which is fundamental for designing specific disease control measures, managing quarantine, and interpreting research data.

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