Fusarium temperatum, a new species from maize closely related to *Fusarium subglutinans*

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Abstract

A novel biological species, *Fusarium temperatum* J. Scauflaire et F. Munaut was described within the *Gibberella fujikuroi* species complex (GFSC), on basis of a robust polyphasic approach. The morphology, the mating compatibilities, the genetic diversity and its relationships with species within the GFSC were analyzed.

Introduction

Among the 5660 *Fusarium* strains belonging to 23 species isolated from maize during a 3-year survey** in Belgium, **276 *Fusarium* strains of an unknown species** were collected (5%). Their elongation factor (*EF-1α*) sequence showed 99% to 100% similarity to the sequence of strain *Fusarium* sp. NRRL 25622 (= MUCL 51714), which has been resolved as phylogenetically distinct from *F. subglutinans* (O'Donnell et al. 2000), although it was reported to be sexually compatible with one of the *F. subglutinans* mating type tester strain (Steenkamp et al. 1999). The aim of the research was to study various traits of those *Fusarium* strains in order to define their taxonomical rank within the GFSC.

Phylogeny

The combined β-tubulin and *EF-1α* datasets consisted of 1184 aligned nucleotide positions. Bayesian analyses resulted in a posterior probability distribution containing 2000 samples per analysis. The initial 1521 samples were discarded and a majority rule consensus tree of the remaining combined samples was produced. Parsimony analysis of the 207 parsimony informative characters resulted in 18 most-parsimonious trees of 745 steps. The majority rule consensus of these trees produced a tree of topology that showed no supported conflicts with that produced by the Bayesian analysis.

In all trees and in the majority rule consensus tree, all strains of *F. temperatum* formed a **strongly supported monophyletic group** (Bayesian posterior probability (PP) = 1; bootstrap (B) = 100). *F. subglutinans* strains were placed in a distinct well-supported clade (PP = 1; B = 100) (Fig. 14). The overall tree topology was similar to those previously presented for the GFSC (O'Donnell et al. 1998), in which NRRL 25622 (= MUCL 51714; described here as *F. temperatum*) was placed in the "American Clade".

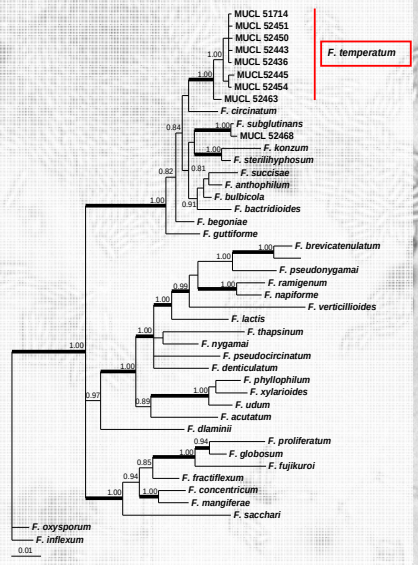
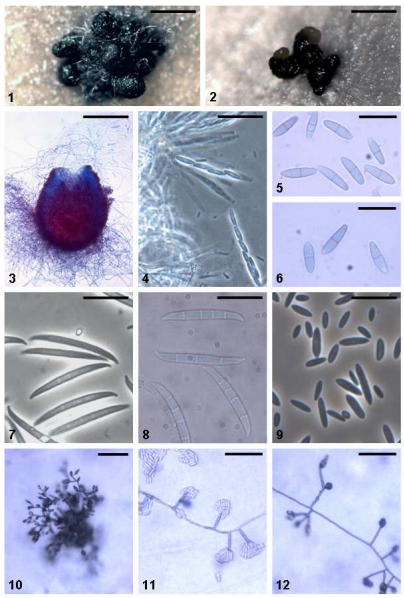


Figure 14. Bayesian inference tree based on partial sequences of β-tubulin and *EF-1α* loci. Values at branch nodes indicate support with posterior probabilities (PP; values ≥ 0.80 shown) and branches in bold = bootstrapping percentages based on maximum parsimony analysis ≥ 70 %. Scale bar represents expected substitutions per site.



Figures 1-12. *Fusarium temperatum* teleomorph from cross of MUCL 52463 X MUCL 52438. **1-2**. Perithecia. Scale bar (SB) = 0.5 mm. **3**. Longitudinal view of perithecium. SB = 0.2 mm. **4**. Asci cylindric, apex with a shallow, refractive ring. SB = 50 µm. **5-6**. Ascospores. SB = 20 µm. *Fusarium temperatum* anamorph from MUCL 52463. **7-8**. Falcate, mostly 4-septate macroconidia. SB = 20 µm. **9**. Ellipsoidal to oval, obovoid unseptate microconidia and fusiform, 1-2 septate microconidia. SB = 20 µm. **10**. Aerial mycelium with erect, branched, polyphialidic conidiophores producing conidia either singly or in false heads. SB = 0.1 mm. **11-12**. Aerial mycelium with monophialides and intercalary phialides producing conidia in false heads. SB = 50 µm.

Figure 13. Dendrogram generated from UPGMA cluster analysis showing the genetic similarities (Dice similarity coefficient) among *Fusarium* species of this study. Support from 1000 bootstrap iterations is indicated for those clusters receiving greater than 70%. →

Interspecies and intraspecies compatibility

After determining the mating-type idiomorph for each *F. temperatum* strain, 5 MAT1-1 and 5 MAT1-2 strains were selected for fertility crosses.

- All 10 strains of *F. temperatum* were unfertile when crossed with the *F. subglutinans*, *F. circinatum*, and *F. konzum* tester strains belonging to the "American Clade".
- The 5 MAT1-1 strains, the 5 MAT1-2 strains and strain NRRL 25622 (MAT1-2) were intercrossed. All 11 strains of *F. temperatum* were fertile as male in these crosses and 5 strains were female fertile. Based on these intra-specific crosses, **MUCL 52463 and MUCL 52438 were selected as reliable female fertile tester strains** of MAT1-1 and MAT1-2 respectively.

According to the mating compatibility testing of the present study, *F. temperatum* represents a **new biological species** in the GFSC, within the "American clade". The relatively high percentage of female fertile strains and the observed mating-type ratio (MAT1-1:MAT1-2 = 17:13) suggested that sexual reproduction may be common in fields (Leslie and Klein 1996).

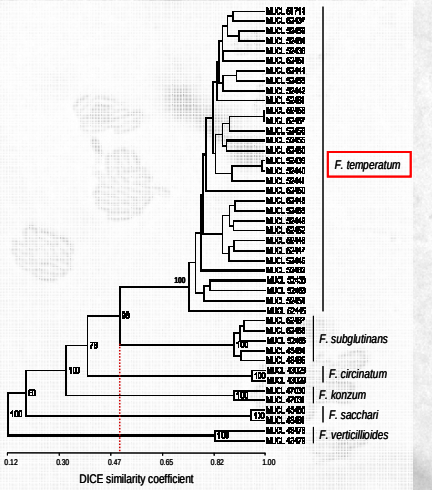
Materials and methods

30 *Fusarium* sp. strains isolated from maize in Belgium were compared with *F. subglutinans* strains from the same origin, with the NRRL 25622 strain, and with other *Fusarium* species of the GFSC. Therefore, we used a **polyphasic approach** based on:

- AFLP study to compare the genetic similarity between *Fusarium* strains.
- Phylogenetic species recognition (parsimony and Bayesian analyses).
- Biological species recognition (sexual crosses).
- Morphological species recognition.

AFLP

The AFLP analysis clustered the *F. temperatum* isolates and the *F. subglutinans* in two sister groups, with a **51% Dice similarity coefficient**. This percentage is comparable to those observed between different mating populations in the GFSC (Zeller et al. 2003). In the *F. temperatum* cluster, genetic similarity among the 31 strains ranged from 74 to 100%, what is consistent with previous descriptions of isolates belonging to the same species within the GFSC (Leslie and Summerell 2006). Tester strains of *F. circinatum*, *F. konzum*, *F. sacchari*, and *F. verticillioides* are included in the 4 other groups. The distinctness of all 6 groups was supported by bootstrap values of 78–100% in 1000 replicates (Fig. 13).



Taxonomy

Fusarium temperatum J. Scauflaire et F. Munaut, sp. nov. (Fig. 1-12)

- **Colonies** on PDA: Aerial mycelium cottony, initially white, later become pinkish-white, rarely tinged violet in the center by the substrate. Pigmentation in reverse slightly pinkish-orange. No chlamydospore nor sclerotia observed.
- **Anamorph**: Macroconidia hyaline, 3-6 septate (mostly 4-septate), falcate, with a beaked curved apical cell and a footlike basal cell; 22-50 µm long, 2-4 µm wide. Microconidia hyaline, 0-2 septate; ellipsoidal to oval, obovoid when unseptate, 3-13 µm long, 2-4 wide; fusiform when 1-2 septate, 11-23 µm long, 3-5 µm wide.
- **Teleomorph**: Perithecia ovoid to obpyriform, superficial, mostly aggregated, nonstromatic, and slightly warty; 215-405 µm high, 170-310 µm wide. Ascospores exuded in a cirrhus, smooth, hyaline, ellipsoidal to oval, 1-2 septate, mostly 1 septate (85%), slightly constricted at the septum; 13-22 µm long, 4.5-6 µm wide.

Conclusion and Perspectives

- Based on the results of this polyphasic approach, the population of *Fusarium* strains isolated from maize in Belgium that were closely related to *F. subglutinans* and the strain NRRL 25622 were described as a new species, ***Fusarium temperatum*** (Scauflaire et al., Mycologia, in press).
- Preliminary pathogenicity assay results confirm the ability of *F. temperatum* to **cause stalk rot and seedling malformation** with a similar virulence than *F. subglutinans* (unpublished data).
- The *F. temperatum*:*F. subglutinans* ratio was very high in Belgian fields (276:9), suggesting that *F. temperatum* apparently competed *F. subglutinans*.
- An interesting hypothesis for the separation of these two species may be based on climate (Moretti et al. 2008). *F. temperatum* are isolated in moderate to cool and wet temperate regions (hence its epithet), while *F. subglutinans* isolates are more common in warmer and dryer regions.
- Finally, given that a culture extract of strain NRRL 25622 was reported to be toxigenic (Sewram et al. 1999), studies are also in progress to elucidate the mycotoxigenic potential of *F. temperatum*.

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