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Existence of *Fusarium nygamai* in the southern region of Sri Lanka and a key for their characterization

W. P. Sapumohotti
Department of Botany, University of Ruhuna, Matara, Sri Lanka.

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ABSTRACT

In the course of survey throughout southern region of Sri Lanka during the period 1999-2002, more than 100 isolates of *Fusarium nygamai* were obtained from a variety of sources including soil and naturally diseased plants. Emphasis is given to cultural features, growth rates, mode of production of micro- and macroconidia, and the presence or absence of polyphialides for the species delimitation. *F. nygamai* is a relatively newly described species and has never been assigned to any of the sections but it appears to have an affinity with the section *Liseola*. Therefore, it is proposed that *F. nygamai* which produces chlamydospores be included in the section *Liseola*. Hence, the absence of chlamydospores should no longer be a characteristic of the species in the section *Liseola*. Description and key to the identification of the species are also included.

Keywords: *Fusarium nygamai*, Liseola, Sri Lanka.

INTRODUCTION

Fusarium is an ubiquitous genus of imperfect fungi widely distributed in soil and organic substrates. Uncountable references have revealed the importance and potential of *Fusarium* as disease causing organisms, especially in the tropics. Some species can also cause human diseases such as onchomycosis, keratomycosis and skin lesions (Jayaraman and Kalyan - asundaram 1990; Joffe 1978; Marasas *et al.*, 1988a; Pitt 2000; Rheeder *et al.* 2002;Zapater 1986). Some *Fusarium* spp. are responsible for disorders in mammals such as Leukoencephalo -- malacia, pulmonaryedema and carcinogenicity due to potent toxins such as fumonisins (Fendohan *et al.*, 2003; Marasas *et al.*, 1988a).

The bulk of fusaria isolated from nature are generally saprophytes or weak parasites because of their ability to remain dormant in bare soils, decayed plants and plant debris for years in the form of chlamydospores, conidia or free-living mycelia. Although there are no reported epidemics caused by *Fusarium* in Sri Lanka, considerable losses have been recorded on banana (Panama wilt caused by *F. oxysporum* f. sp. *cubense*) and several other crops (Gamini *et al.*, 1999; Jeyanandarajah 1991; Sapumohotti and Padmanatha 1997a; 1997b) due to *Fusarium* infections. The pathogenic potential of *Fusarium* should not be underestimated as it has a great ability to mutate both in culture and nature (Britz *et al.*,2002; Salleh and Sulaiman 1984).

The taxonomy of *Fusarium* began with the description of the genus by Link (1809) based on the presence of fusiform non-septate spores borne on a stroma. However, the publication of Die Fusarien became the foundation of the present system of classification (Wollenweber and Reinking 1935). Later, Booth (1971) introduced a system of classification based on the morphology of the conidiogenous cells and conidium ontogeny. However, many workers found that this classification was too detailed and difficult to follow. Thus, efforts are being made all over the world to simplify the classification system. This has led to the existence of several different systems of classification which regretfully, are still not satisfactory for the identification of all *Fusarium* species.

The most acceptable part of the classification has been the separation of the genus into sections or groups which were defined by Booth (1971) as aggregations of related species. Nelson *et al.*, (1983) separated each section based on 1) the presence or absence of microconidia. 2) the shape of the microconidia, 3) the presence or absence of chlamydospores. 4) the location of the

Chlamydospores; intercalary or terminal, 5) the shape of the macroconidia, and 6) the shape of the basal cells or the foot cells of the macroconidia. One of the sections recognized by all *Fusarium* taxonomists is *Liseola* (Britz., 2002; Wollenweber and Reinking 1935). Wollenweber and Reinking (1935) included 3 species and 3 varieties in the *Liseola* section (Table 1). Snyder and Hansen (1945), however, reduced the number to a single species *i.e. F. moniliforme* Sheldon emend. Snyder and Hansen., while Gerlarch and Nirenberg (1982) increased the number to 9 species and 5 varieties. Booth (1971) recognized one species *i.e. F. moniliforme* with one variety *subglutinans* but Nelson *et al.*,(1983) recognized 6 species within the Section. According to Booth (1971), the characteristics of the species in this section are based on a) microconidia formed in chains or false heads, b) microconidia spindle to ovoid in shape, c) macroconidia slender with constricted apical cell and pedicellate basal cell, d) chlamydospores absent, and e) cultures brownish to orange cinnamon.

Table 1. *Fusarium* species in the section *Liseola* by different taxonomic systems

Wollenweber and Reinking (1935)	Boooth (1971)	Gerlarch and Nirenberg (1982)	Nirenberg (1983)
F.monilifome *F.moniliforme* Var.*anthophilum* *F.moniliforme* var. *minus* *F.moniliforme* *var.subglutinans* *F. lactis* *F. Neoceras*	*F .moniliforme* *F. moniliforme* var. *Subglutinans*	*F. verticillioides* *F. fujikuroi* *F. poliferatum* var. *proliferatum* *F. proliferatum* var. *minus* *F. annulatum* *F. sacchari* var.*sacchari* *F. sacchari* *var.subglutinans* *F. sacchari* var. *elongatum* *F. succisae* *F. anthophilum* *F. lactis* *F.neoceras*	*F.moniliforme* *F .proliferatum* *F. subglutinans* *F. anthophilum* *F. annulatum* *F.succisae*

F. nygamai, a relatively newly described species of considerable taxonomic interest because it cannot be included in any of the existing sections of the genus (Abbasher and Sauerborn 1992; Burgess and Trimboli 1986). Although it appears to have some affinity with section *Liseola* it is excluded from section *Liseola* due to formation of chlamydospores (Burgess and Trimboli 1986; Nelson *et al.*,1992).

The shape of the macroconidia formed in sporodochia is a key criteria in *Fusarium* Taxonomy. (Booth 1971; Nelson *et al.*,1983). This criterion, however, cannot be used to differentiate *F. nygamai* from species in the section *Liseola* and *Elegans* because there is little variation in shape of the macroconidia between species in these sections and *F. nygamai.* Thus the key criteria for the identification of *F. nygamai* are the formation of microconidia in short chains and false-heads, and the formation of chlamydospores. In this paper, *F. nygamai* from the southern region of Sri Lanka are described following a classification system devised by Burgess and Trimboli (1986).

MATERIALS AND METHODS

During the survey throughout Southern region of Sri Lanka between1999-2002, more than 100 isolates of *F. nygamai* have been isolated. These isolates were collected from a variety of sources including soil and naturally diseased plants (maize, mango, orchids, rubber, tea, rice, watermelon, chili, banana, citrus, guava and bean). Single spore isolations were carried out following the method of Hansen and Smith (1932) and incubated under standard incubation conditions (Salleh and Sulaiman 1984). The delimitation of the species was carried out following diagnostic characteristics outlined by Burgess and Trimboli (1986). Single-spored cultures grown on potato sucrose agar (PSA) plates (Salleh and Sulaiman 1984), KCl-agar medium (Nelson *et al.*,1983) and soil extract agar (Booth 1971) were used to determine the ability of cultures to form microconidia in chains and to stimulate the production of chlamydospores, respectively. Growth rates of monoconidial cultures on PSA were recorded as mean diameters of the colonies on the fourth day of incubation (Booth 1971). The Methuen handbook of colour (Kornerup and Wanscher 1978) was used as the colour reference.

RESULTS AND DISCUSSION

Burgess and Trimboli (1986) was first isolated *F. nygamai* from the roots of grain sorghum in Australia. Subsequently, it was isolated from the roots of French bean (*Phaseolus vulgaris* L.) and grassland soil in eastern Australia. This species also have been reported from the Republic of South Africa, the Republic of Transkei, Thailand, Malaysia, Puerto Rico and few other countries in

the world (Burgess and Trimboli 1986; Fendohan *et al.*, 2003; Marasas *et al.*, 1988b; Sapumohotti and Salleh 1991; 1992). However, its existence and distribution in Sri Lanka is not known.

Isolates of a species similar in some respects to *F. moniliforme* have been collected from all over the world. Some of them have been identified as *F. nygamai* by Burgess and Trimboli (1986) following the aboriginal name of the host where they were first isolated. *F. nygamai* has many similar characteristics to *F. moniliforme* except for the production of chlamydospores which are absent in all species in the section *Liseola*. It has been referred to as the "short-chain type" of *F. moniliforme* by Nelson *et al.*,(1983). At present, *F. nygamai* has not been assigned to any of the section since its characters are intermediate to those of *F. oxysporum* in the sections *Elegans* and *Liseola*. It is similar to *F. oxysporum* (section *Elegans*) due to the presence of falcate to almost straight macroconidia and the production of chlamydospores. However, *F. oxysporum* produces its microconidia only on false heads and never in chains.

***F. nygamai.* Burgess and Trimboli.**

Initially mycelium floccose, white becoming dull to violet, hairy and felted with slight growth zonation. Orange to purple pigmentation usually appears at the center and at the outer ring. Growth rate 2.0 5.3 cm.

Microconidia abundant, produced in short chains (upto 16 conidia) or false heads on polyphialides and simple phialides formed on branched conidiophores. They are 0-septate, 4.4 17.6 X 2.4 3.4 m, mostly clavate, elliptical or oval with a flattened base (Fig. 1).

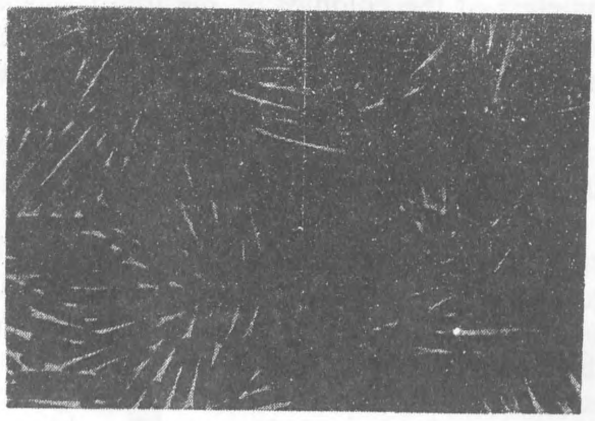

Fig. 1

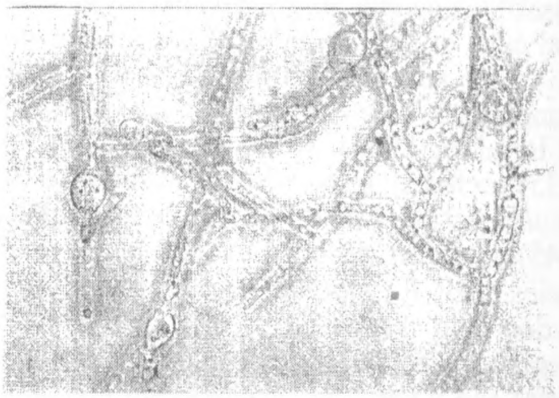

Fig. 2

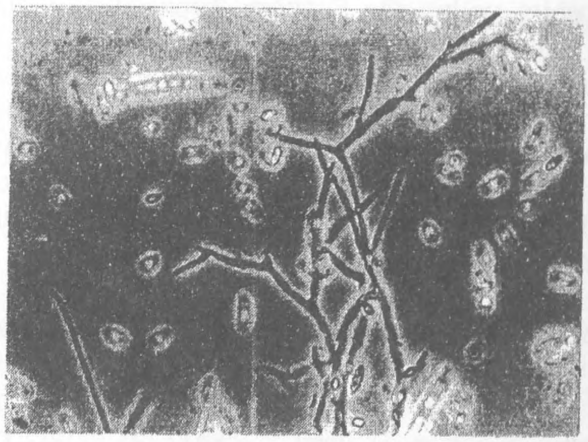

Fig. 3

Figs. 1-3 *Fusarium nygamai*
1. Macro- and microconidia
2. Polyphialides
3. Chlamydospores (All X 560).

Macroconidia are slender, falcate, short to medium length and up to 5-septate (Fig. 1). They are formed in violet sporodochia or on branched lateral conidiophores (Fig. 3); 3-septate: 10.2 18.2 X 2.5 4 m, 5-septate: 19.4 41.5 X 3.0 3.2 m. The septation is not very clear; thin walled with a short tapered apical cell and foot shaped base.

Chlamydospores rare to abundant, solitary (Fig. 2), in short chains or clumps, terminal or intercalary with smooth-walled. However, in certain isolates, the presence of chlamydospores are hard to be observed since they also produce hyphal swellings which closely resemble chlamydospores. Chlamydospores are usually thick-walled and granulated with dense cytoplasm whereas hyphal swellings are hyphae that swelled,

probably, due to certain stress factors. Therefor one must observe very carefully mature cultures (up to 3 weeks) on nutrient poor media e.g. soil extract agar to distinguish between chlamydos - -pores and hyphal swellings.

I herewith propose that *F. nygamai* be included in the section *Liseola* due to many similarities of this species with all other members of the section. Therefore, the description of *Fusarium* species in the section should be extended to include the presence of chlamydopores, at least in one species. This amendment is not too drastic since the original description of the section has subsequently been changed considerably. For example, chains of microconidia are no longer diagnostic characters of the section.

Key to the delimitation of *Fusarium* species in the section *Liseola* in Sri Lanka.

1. Microconidia form on monophialides only .....*F. moniliforme*
   Microconidia form on mono- and polyphialides.....

2. Chlamydospores absent.....
   F. proliferatum
   Chlamydospores present.....
   F. nygamai

There are many publications concerning the ability of *F. moniliforme* to cause diseases of plants, animals and humans, and to produce hazardous mycotoxins but during that period, researchers were not aware of the identity of *F. nygamai* which resembles *F. moniliforme* very closely. This is not surprising since the usual procedure for the identification of *F. moniliforme* at that time was to observe the cultures for the production of microconidia in chains in younger cultures, regardless whether the microconidia were produced on polyphialides and whether the chlamydospores were present or absent in older cultures. Polyphialides and chlamydospores produced by *F. nygamai* would only be observed in cultures of at least 21 day old (Abbasher and Sauerborn 1992; Marasas *et al.*, 1988b). Researchers working with *Fusarium* species in the *Liseola* section, especially *F. moniliforme*, should always continue to monitor cultures on media with low nutritional status to be confident that the cultures do not produce polyphialides and Chlamydospores after 3-4 weeks of incubation.

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REFERENCES

Abbasher A A and Sauerborn J 1992 *Fusarium nygamai*: a potential bioherbicide for *Striga hermonthica* control in sorgum. Biological Control. 2: 291-296.

Booth C (eds.) 1971 The Genus *Fusarium*, Commonwealth Mycological Institute, Kew, Surrey, England.

Britz H, Steenkamp E T, Coutinho T A, Wingfield B D, Marasas W F O and Wingfield M J 2002 Two new species of *Fusarium* section *Liseola* associated with mango malformation. Mycopathologia 94: 722-730.

Burgess L W and Trimboli D 1986 Characterization and distribution of *Fusarium nygamai*, sp. nov. Mycologia. 78: 223-229.

Fendohan P, Hell K, Marasas W F O and Wingfield M J 2003 Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. African J. Biotech. 2: 570-579.

Gamini W A, Sapumohotti W P, de Soyza, I J and Alahakoon P W 1999 Status of Fusarium wilt of banana (Panama disease) in Sri Lanka. International Seminar and Workshop on the Banana Fusarium Wilt Disease, Genting Highlands Resort, Malaysia.

Gerlarch W and Nirenberg, H (eds.)1982 The Genus *Fusarium*: A Pictorial Atlas, Mitt. Biol. Bundesanst. Land. Forstwirtech, Berlin.

Hansen H N and Smith R E 1932 The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopathol. 22: 953-964.

Jayaraman P and Kalyanasundaram I 1990 Natural occurrence of toxigenic fungi and mycotoxins in rice bran. Mycopathologia. 110: 81-85.

Jeyanandarajah P 1991 Seed-borne fungi of ome important crops grown in Sri Lanka. Seed Sci. and Technol. 19: 633-646.

Joffe A Z 1978 *Fusarium poae* and *Fusarium sporotrichioides* as principal causal agents of alimentary toxic aleukia. In: Wylle T D and Morehouse L G. (ed.) Mycotic Fungi,

Mycotoxins and Mycotoxicosis, Berlin. pp. 21-86.

Kornerup A and Wanscher J H (eds.) 1978 Metheun Handbook of Colour, London, U.K.

Link H F 1809 Observations in ordines plantarum naturales. *Mag Ges Naturforsch Freunde*, Berlin 3: 1-10.

Marasas W F O, Jaskiewiez K, Venter F S and van Schalkwyk D J 1988a *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. South African Medical Journal 74: 110-114.

Marasas W F O, Rabie C J, Lubben A, Nelson P E, Toussoun T A and van Wyk P S 1988b *Fusarium nygamai* from millet in South Africa. Mycologia 80: 263-266.

Nelson P E, Plattner, R D, Shackelford D D and Desjardins A E 1992 Fumonisin B1 production by *Fusarium* species other than . *Fmoniliforme* in section Liseola and by some related species. Appl. Environ. Microbiol. 58: 984-989.

Nelson P E, Toussoun T A and Marasas W F O (eds.) 1983 *Fusarium* Species: An Illustrated Manual for Identification, University Park, Pennsylvania State University.

Pitt J I 2000 Toxigenic fungi: Which are important? Med. Mycol. 38: 17-22.

Rheeder J P, Marasas W F O and Vismer H F 2002 Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68: 2101-2105.

Salleh B and Sulaiman B 1984 Fusaria associated with naturally diseased plants in Penang. J. Pl. Prot. Tropics. 1: 47-53.

Sapumohotti W P and Padmanatha H G C 1997a Evaluation of media on the generation of nitrate reduction mutants in *Fusarium oxysporum* f. sp. *cubense*, the causal organism of Panama disease in banana. 53rd Annual Sessions of SLAAS, Sri Lanka (Abstract).

Sapumohotti W P and Padmanatha H G C 1997b Genetic diversity of *Fusarium oxysporum* f. sp. *cubense*, the causal organism of Panama disease in banana. 53rd Annual Sessions of SLAAS, Sri Lanka (Abstract).

Sapumohotti W P and Salleh B 1991 Frequency of sectoring of *Fusarium nygamai* causing reddish brown rot of asparagus. UNESCO National Workshop, Serdang, Malaysia (Abstract).

Sapumohotti W P and Salleh B 1992 Nitrate reduction mutants of *Fusarium nygamai* causing reddish brown rot of asparagus. J. Pl. Prot. Tropics. 9: 187-194.

Snyder W C and Hansen H N 1945 The species concept in *Fusarium* with reference to Discolor and other sections. Amer. J. Bot. 32: 657-666.

Wollenweber H W and Reinking O A (eds.) 1935 Die Fusarien ihre Beschreibung, Schwadwirkung und Kekampfung, Berlin, Paul Parey.

Zapater R C 1986 Opportunistic fungus infection-*Fusarium* infections-Keratomycosis by *Fusarium*. Jpn. J. Med. Mycol. 27: 68-69.