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Fusarium globosum from subtropical Japan and the effect of different light conditions on its conidiogenesis

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The second report of *Fusarium globosum* is based on strains isolated from wheat in Ishigaki Island, Okinawa, in subtropical Japan. Morphological features of the Japanese isolates are described. These strains show different reactions in conidiogenesis to light conditions. Under continuous BLB light, falcate sporodochial conidia are typically induced, but production of aerial globose conidia is suppressed. In two of the strains, clavate conidia became longer under BLB light. Application of both, complete darkness and continuous BLB light, is recommended as standard light conditions to culture *Fusarium* isolates.

Key Words——BLB light; conidiogenesis; *Fusarium globosum*; subtropical Japan; *Triticum aestivum*.

Together with other species of *Fusarium*, strains of *F. globosum* Rheeder, Marasas & Nelson were isolated from wheat culms with a high frequency, during a survey on the occurrence of microfungi from gramineaceous substrates in subtropical Japan. The species reacted differently in conidiogenesis when cultured with or without BLB light illumination. In this paper, *F. globosum* from subtropical Japan is described and illustrated as the second report after the first by Rheeder et al. (1996) from South Africa. Methods to study morphology of the species are also presented and discussed.

Materials and Methods

Collection of study material Wheat plants were obtained at the Okinawa Branch, Tropical Agriculture Research Center (TARC; present name: Japan International Research Center for Agriculture Sciences, JIRCAS), MAFF, Ishigaki, Okinawa Pref., Japan (24°16.7′N, 123°53.0′E) on May 10, 1991, which were cultivated in an experimental field for breeding (variety: Norin 61). At the full ripening stage, basal parts of healthy wheat culms (5 cm in length) were cut, air-dried, and brought back to the laboratory.

Washing method and fungal isolation Five wheat culms were cut into halves longitudinally and each half was washed by a modified washing procedure (Tokumasu, 1980; Aoki et al., 1990). Sterilized 0.005% Aerosol OT aqueous solution (di-*iso*-octyl sodium sulfosuccinate) was used as the washing detergent. The washing procedure was repeated ten times. At the end, the material was rinsed with sterilized distilled water for three times. Washed culms were placed on sterilized filter paper in 9-cm Petri dishes to remove excess water from the surface and dried over night. Then, each of the cut culms was placed onto a half-strength corn meal agar plate and incubated to examine fungal growth.

These plates were examined microscopically three times: after 1, 2 and 4 wk. Fungi, including species of *Fusarium*, appeared on and around the wheat culms. They were isolated as pure cultures and identified. Some fungal species were also identified directly by preparing microscopic mounts from the incubated plates. The occurrence of individual fungi was evaluated by percentage frequency, calculated by the following equation: Percentage frequency of the fungus (%)=(number of culms on and around which the fungus was detected) / 5 (number of culms examined) × 100.

Morphological examination of *Fusarium* species Isolates of *Fusarium* were examined for cultural and microscopic characters. Morphological features were compared on different culture media and under different light regimes to assure a precise identification of the species (Nirenberg, 1976, 1990). For the examination of such characters as growth rate, colony color and odor, strains were grown on potato dextrose agar (PDA; Difco) in 9 cm plastic Petri dishes. Microscopic characters were examined of cultures on synthetic low nutrient agar (SNA) with a piece of ca. 1 × 2 cm sterile filter paper placed on the cooled agar surface. Cultures were incubated for 14 d at 25°C in complete darkness, under continuous BLB (Black Light Blue; near-UV, peak wavelength: 365 nm) light illumination (Toshiba FL20S BLB20W;

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Seemüller, 1968; Nirenberg, 1990), and under an alternating 12 h darkness/12 h BLB light cycle. Unless stated otherwise, mean and standard deviation (S.D.) in size of individual conidial types are derived from the measurement of 30 conidia, randomly chosen from a culture under each of the above conditions. Colors cited are given according to the Methuen handbook of colour (Kornerup and Wanscher, 1978).

Selected strains of examined *Fusarium* species were deposited in MAFF (Genetic Resources Center, National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki, Japan) and in BBA (Institut für Mikrobiologie, Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany).

Results

Fungal occurrence on wheat culms Fungal species isolated from the basal 5-cm culms of wheat from Ishigaki Island are shown in Table 1, with their frequencies of occurrence. In total, 15 species of microfungi were isolated from the culms. The dominant species (80%) were *Cladosporium cladosporioides* (Fres.) de Vries, *C. oxysporum* Berk. & Curt. and *Fusarium incarnatum* (Rob.) Sacc. (=*F. semitectum* Berk. & Rav. var. *majus* Wollenw.), of which the latter two are known as tropical species (Ellis, 1971; Gerlach and Nirenberg, 1982). Two species of *Fusarium*, i.e., *F. globosum* and *F. poae* (Peck) Wollenw., and *Bipolaris sorokiniana* (Sacc.) Shoem. were identified as frequent fungi (60%), followed by yet another species of *Fusarium*, i.e., *F. proliferatum* (Matsushima) Nirenberg, as well as two species of *Nigrospora* and *Alternaria alternata* (Fr.) Keissler respecting their order of frequency. Among the 15 detected species of microfungi, 6 were identified as *Fusarium*, the frequencies of which varied between 20 and 80%.

Table 1. Fungal occurrence from basal 5-cm culms of wheat, collected at an experimental field of TARC in Ishigaki Island, Okinawa, 10 May 1991.

Species	% frequency[a)]
Cladosporium cladosporioides	80
Cladosporium oxysporum	80
Fusarium incarnatum	80
Fusarium globosum	**60**
Fusarium poae	60
Bipolaris sorokiniana	60
Fusarium proliferatum	40
Nigrospora oryzae	40
Nigrospora sphaerica	40
Alternaria alternata	40
Fusarium graminearum	20
Fusarium oxysporum	20
Cladosporium tenuissimum	20
Stemphylium vesicarium	20
Phoma sp.	20

a) Percentage frequency of occurrence.

Fungal description From the wheat culms examined, *F. globosum* was isolated as a frequent colonizer (60%). The fungus is described and illustrated here as a new record for Japan, as well as the second report of the species in the world.

Fusarium globosum Rheeder, Marasas & Nelson, Mycologia **88**: 509. 1996. Figs. 1–32

Colony growth on PDA 3.75–6.25 mm per d at 25°C in the dark. Colony margin entire to undulate. Aerial mycelium entire white (1A1), sometimes becoming purplish white (14A2), abundantly developed, loosely to densely floccose, sometimes funiculose. Reverse in white (1A1), orange white (5-6A2) to pale orange (5-6A3) shades. Hyphae 1–5(–7) μm wide. Sclerotia absent. Odor absent or very faintly sweet. Sporulation on SNA starting quickly in the aerial mycelium and on conidiophores arising directly from the agar surface. Aerial conidiophores at first unbranched, becoming verticillately or sympodially branched. Conidiogenous cells on the aerial conidiophores when producing clavate to ovoid conidia monophialidic, some proliferating sympodially to become polyphialidic, up to 30 μm long and 1.7–4.7 μm wide; those producing globose conidia often remain holoblastic. Conidia borne in the aerial mycelium of two types; 1) ovoid to clavate, then with a truncate base, 0(–1)-septate, hyaline, formed from the tips of phialides mostly in false heads or in short chains, but sometimes forming long chains of up to 29 conidia, giving the culture a powdery appearance, 0-septate conidia: measuring 3.0–17.0 × 1.2–3.7 μm in complete darkness, 5.4–17.7 × 1.7–3.7 μm under BLB light; 2) globose to subglobose with a hilum, sometimes pyriform, 0(–2)-septate, very rarely cruciately septate, hyaline, formed singly or seldom successively on the tip of simple or branched conidiophores, some forming a botryose cluster, measuring 8.6–15.0 × 8.6–14.3 μm in the complete darkness and 7.6–16.2 × 5.4–15.5 μm under BLB light. Both types of conidia frequently formed on the same conidiophores. If sporodochia are produced at all, they appear typically under BLB illumination on the agar-surface or submerged in the agar after 10 d. Sporodochial conidiogenous cells mostly monophialidic, some proliferating sympodially to become polyphialidic, up to 15 μm long, 2–3.5 μm wide, formed on loosely to densely branched short conidiophores. Sporodochial conidia long fusiform to falcate, curved with an acuate apical cell and a distinct basal foot cell, (1–)3–5(–7)-septate, hyaline; 3-septate conidia: measuring 27.8–52.9 × 2.5–3.7 μm in complete darkness, 31.7-61.7 × 2.7–4.4 μm under BLB light, and 5-septate conidia: 43.3–67.4 × 2.7–4.4 μm in complete darkness, 45.0–72.6 × 2.5–4.7 μm under BLB light. Chlamydospores absent.

Strains examined: MAFF 237511=BBA 69019, MAFF 237512=BBA 69017, MAFF 237513=BBA 69018, isolated from *Triticum aestivum* L., collected by T. Aoki, at an experimental field of the Okinawa Branch

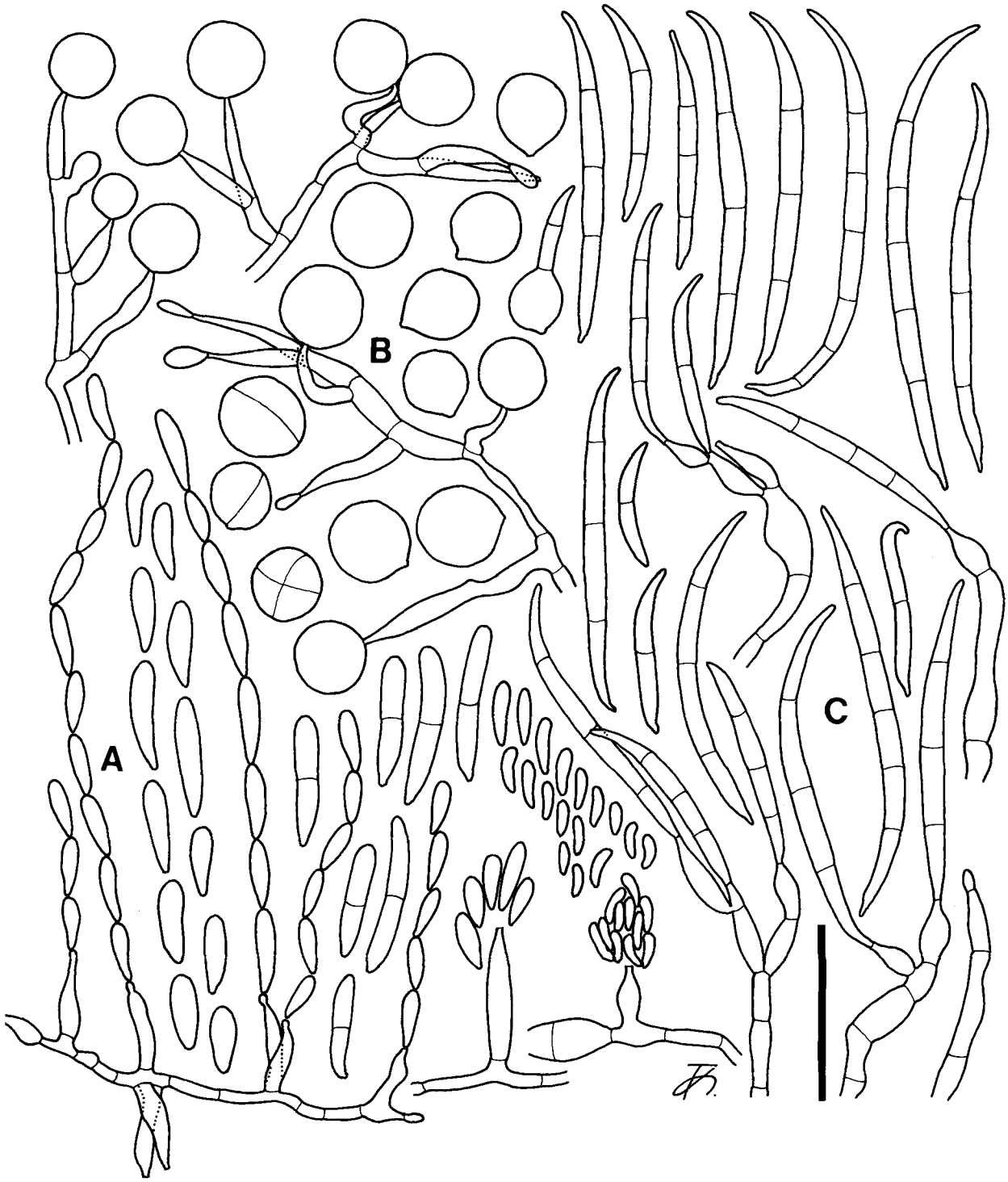


Fig. 1. Japanese isolate of *Fusarium globosum* (MAFF 237512) cultured on SNA under continuous BLB light.
A. Aerial clavate conidia produced from phialides on the aerial mycelium forming conidial chains and false heads. B. Aerial globose conidia formed on simple or branched conidiophores. C. Long fusiform to falcate septate sporodochial conidia, with an acuate apical cell and a foot-like basal cell, formed from phialides on simple or branched conidiophores. Scale bar: 25 μm.

of TARC, Ishigaki Isl., Okinawa Pref., Japan, 10 May 1991.

Effect of BLB illumination on the conidiogenesis of *Fusarium globosum* Conidial production of *F. globosum* varied in strains and under the light conditions applied. Comparison of morphology and conidiogenesis of the strains was made under three different illumination conditions, i.e., in complete darkness, under continuous BLB light

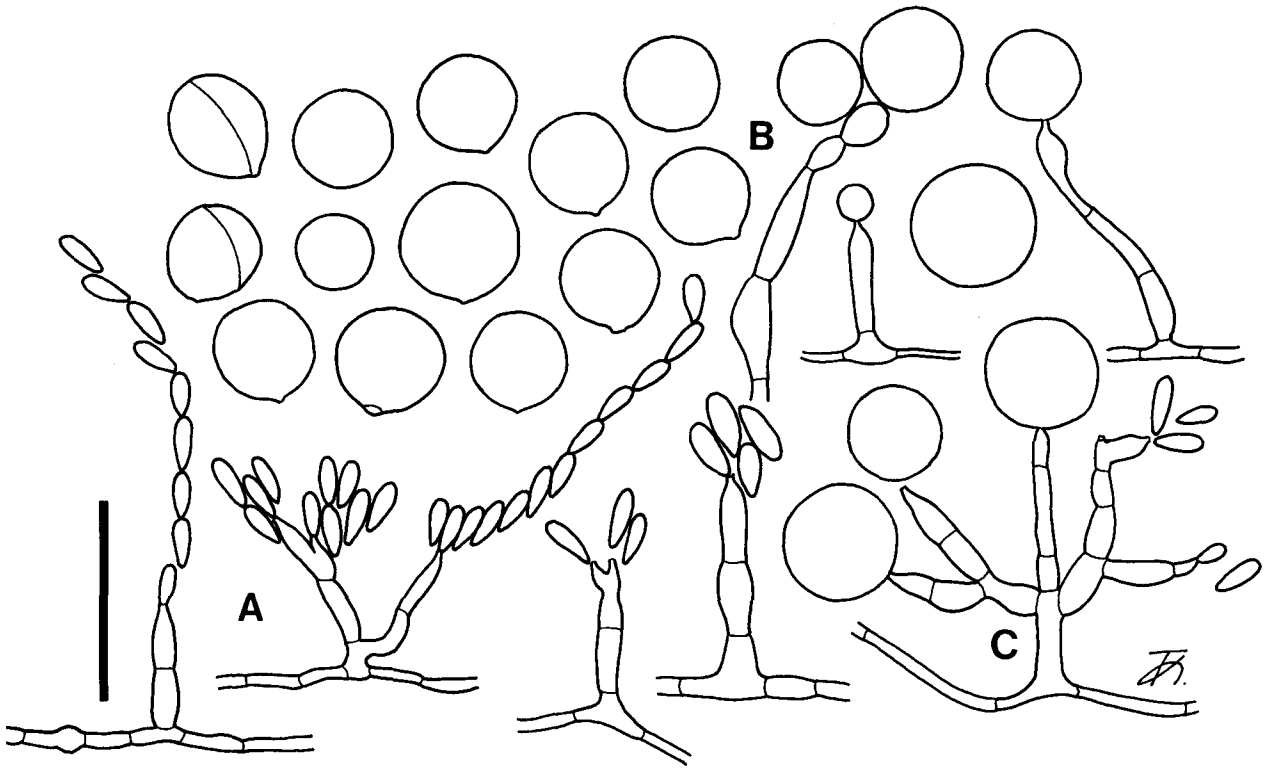
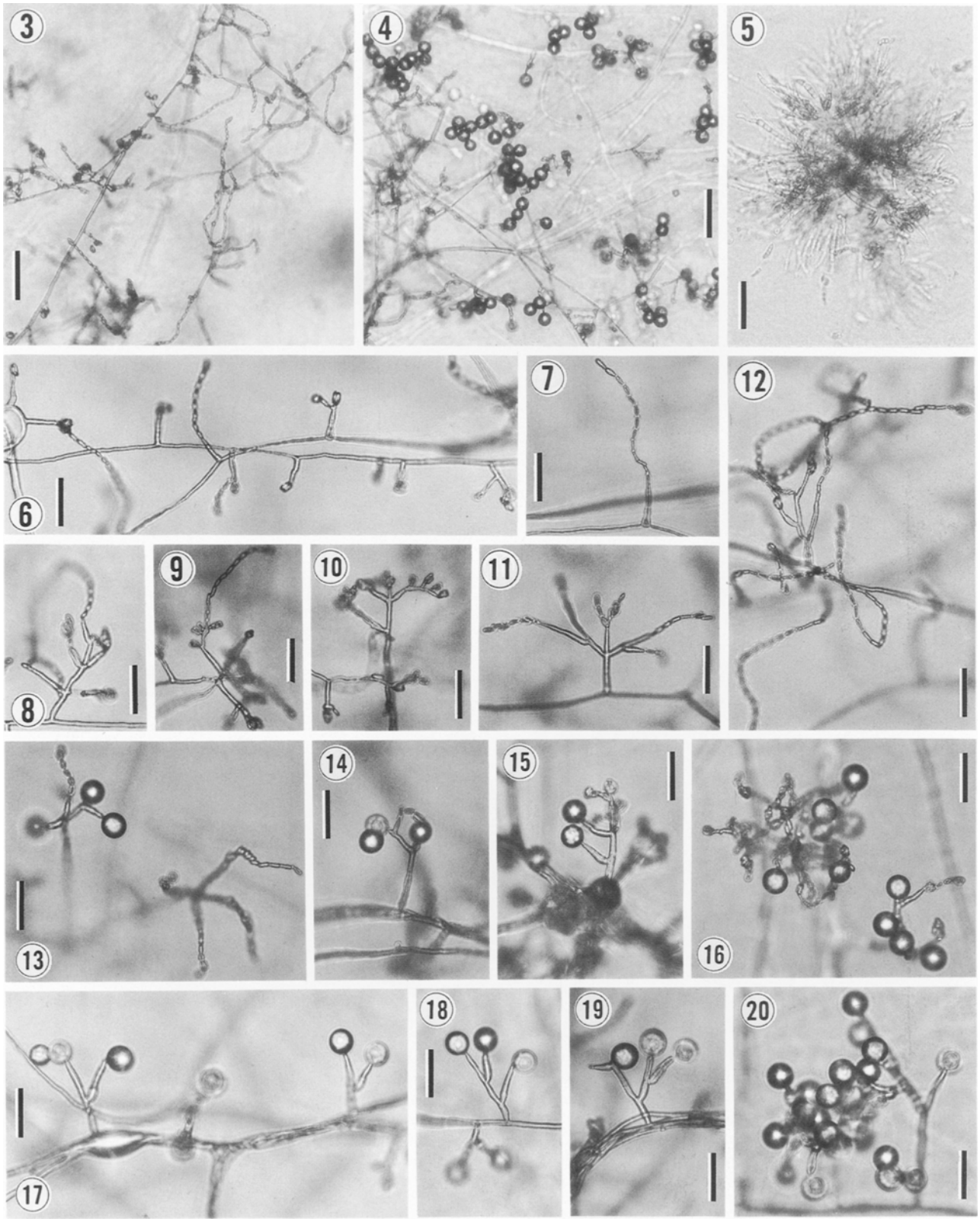


Fig. 2. Japanese isolate of *Fusarium globosum* (MAFF 237512) cultured on SNA in complete darkness.
A. Aerial clavate conidia produced from phialides on the aerial mycelium forming conidial chains and false heads. B. Aerial globose conidia formed on simple or branched conidiophores. C. A branched conidiophore showing production of both clavate and globose conidia from its branches. Scale bar: 25 μm.

and under an alternating 12 h darkness and 12 h BLB light cycle (Table 2). The maximal length of conidial chains, which were generally quite short, varied from strain to strain and with the light conditions used. Strain MAFF 237511 produced relatively short conidial chains of up to 7 to 16 conidia under these light conditions. In strain MAFF 237512, however, conidial chains were longer, i.e., up to 26, 29 and 25 conidia in darkness, under alternating illumination and under continuous BLB light, respectively. In MAFF 237513, the longest chain consisted of 28 conidia was formed in complete darkness, but conidial chains became shorter under continuous BLB light and under alternating illumination (up to 16 and 14, respectively).

The most striking effect of the light conditions on conidiogenesis was observed with three different conidial types induced in the strains of *F. globosum*. They produce typically (1) clavate aerial conidia, (2) globose aerial conidia, and (3) falcate sporodochial conidia. In conidiogenesis of the globose and the falcate conidia, opposite reactions of the strains to BLB illumination were observed. Globose conidia were formed abundantly in darkness by all strains, but were suppressed under BLB light. Strain MAFF 237511 produced no globose conidia under BLB light. In MAFF 237512 and MAFF 237513, they were also formed under BLB light, but were reduced in number markedly when compared with those produced in darkness. When cultured under alternating illumination, the strains showed intermediate expression in conidiogenesis between those when cultured in complete darkness and under continuous BLB light. Under alternating illumination, MAFF 237512 and MAFF 237513 still produced abundant globose conidia, but in MAFF 237511 only 4 were detected in a culture.

Figs. 3–20. Views of the Japanese isolates of *Fusarium globosum* cultured on SNA with a filter paper under a low power of the microscope.
3. Aerial mycelium showing production of clavate conidia in false chains and in false heads. 4. Aerial mycelium showing production of globose conidia. 5. A sporodochium produced on the agar surface. 6-12. Simple or branched aerial conidiophores producing clavate conidia from phialides in false chains and in false heads. 13–16. Production of clavate and globose conidia from branches of the same conidiophores. 17–19. Aerial conidiophores, showing production of globose conidia from the tips of conidiogenous cells. 20. A botryose cluster of globose conidia produced on a conidiophore. Figs. 3–6, 8, 15, 17–20 from MAFF 237511, Figs. 7, 11–14, 16 from MAFF 237512, Figs. 9, 10 from MAFF 237513. Figs. 5, 7–14 from cultures under BLB light, Fig. 16 under alternating light, and Figs. 3, 4, 6, 15, 17–20 in complete darkness. Scale bars: 50 μm in Figs. 3–5, and 25 μm in Figs. 6–20.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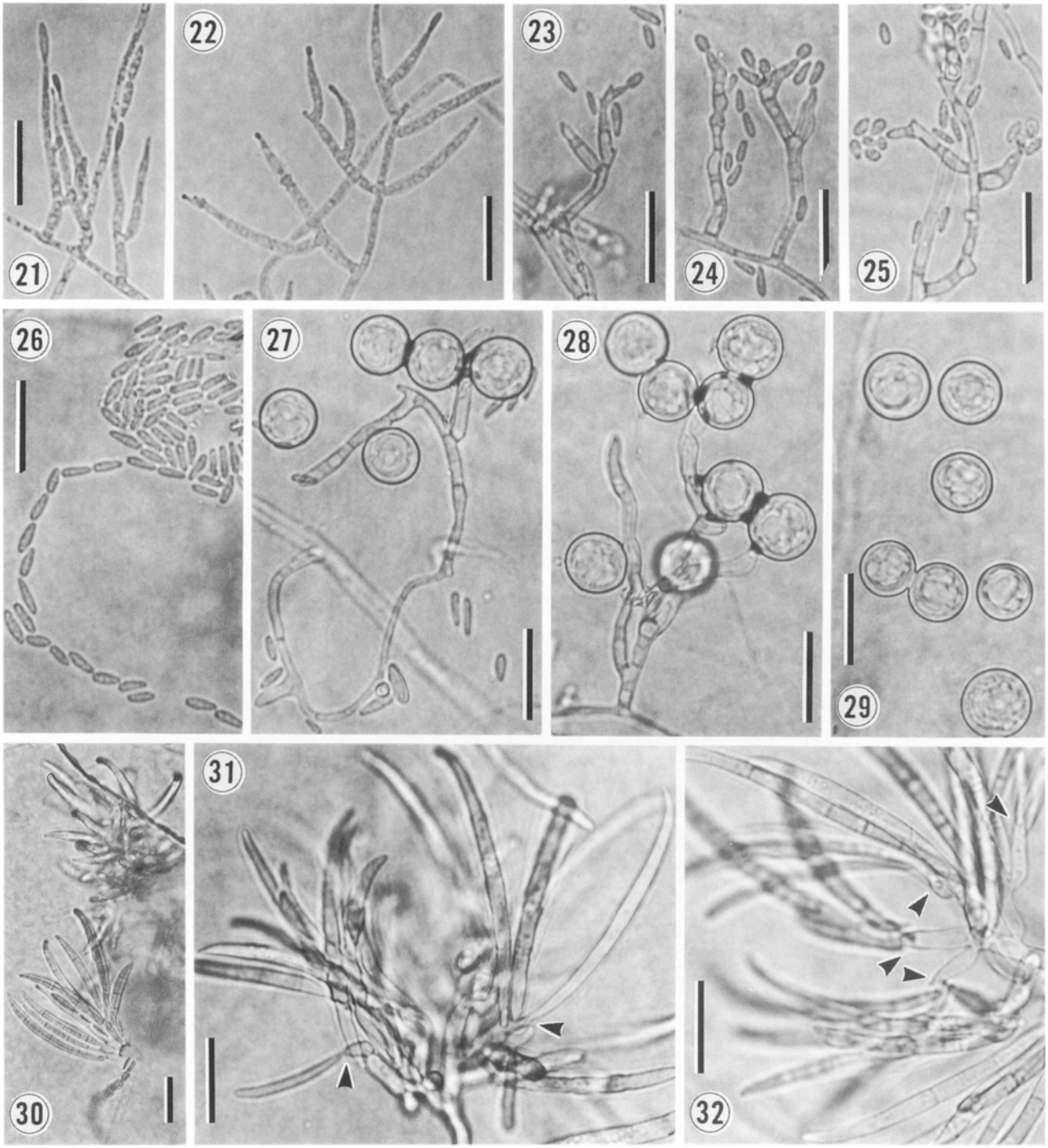

Figs. 21–32. Japanese isolates of *Fusarium globosum* cultured on SNA with filter paper.
21–25. Simple, branched or proliferating aerial conidiophores, showing production of clavate conidia in chains and in false heads. 26. Aerial clavate conidia. 27, 28. Aerial globose conidia on branched conidiophores. 29. Aerial globose conidia. 30. Part of sporodochium forming falcate and septate conidia. 31, 32. Production of sporodochial conidia from the phialides (arrowheads). Figs. 23–30 from MAFF 237511, and Figs. 21, 22, 31, 32 from MAFF 237513. Figs. 25, 27–29 from cultures in complete darkness, and Figs. 21–24, 26, 30–32 from those under BLB light. All scale bars: 20 μm.

Sporodochial conidia, on the contrary, were not observed in MAFF 237512 and MAFF 237513 in complete darkness but were produced under BLB light and under alternating illumination. Micromorphology of these strains in complete darkness was observed as a combination of clavate and globose conidia only (Fig. 2). In MAFF 237511, falcate sporodochial conidia were formed under all conditions, although only 9 conidia with 5 septa were

Table 2. Comparison of morphology and conidiogenesis of *F. globosum* cultured under three different light conditions: in complete darkness (D), under continuous BLB light (B) and under an alternating light cycle, i.e., in 12 h darkness/12 h BLB light (D/B).

Strains	Numbers of conidia in chains and microscopic dimensions (μm): range (average ± S.D.) (length × width)					
	MAFF 237511		MAFF 237512		MAFF 237513	
Conidial chain						
Complete darkness (D)	up to 16		up to 26		up to 28	
Alternate light cycle (D/B)	up to 7		up to 29		up to 14	
BLB light (B)	up to 14		up to 25		up to 16	
Aerial conidia: Clavate (0-septate)						
(D)	4.7–17.0 × 1.2–2.7 (8.1 ± 3.0 × 2.2 ± 0.40)		3.9–7.4 × 1.5–3.0 (5.7 ± 0.95 × 2.3 ± 0.32)		3.0–7.9 × 1.5–3.7 (6.0 ± 1.3 × 2.5 ± 0.40)	
(D/B)	3.9–14.5 × 1.2–3.0 (7.9 ± 2.4 × 2.3 ± 0.41)		4.4–11.8 × 1.5–3.7 (8.0 ± 1.7 × 2.4 ± 0.56)		3.0–15.0 × 1.5–3.0 (7.8 ± 2.2 × 2.4 ± 0.41)	
(B)	6.4–13.0 × 1.7–3.2 (8.6 ± 1.4 × 2.6 ± 0.33)		5.4–15.5 × 1.7–3.7 (9.0 ± 2.6 × 2.6 ± 0.37)		5.7–17.7 × 2.0–3.0 (9.9 ± 2.2 × 2.6 ± 0.27)	
Aerial conidia: Globose						
(D)	10.1–13.5 × 9.1–12.8 (11.7 ± 0.76 × 11.4 ± 0.86)		8.6–15.0 × 8.6–14.3 (12.5 ± 1.4 × 12.4 ± 1.3)		10.3–13.8 × 10.1–14.0 (12.3 ± 0.95 × 12.2 ± 1.0)	
(D/B)	5.9–12.5 × 5.2–12.3 (10.2 ± 2.58 × 10.0 ± 2.82)[b]		9.3–14.8 × 7.6–14.3 (11.3 ± 1.3 × 10.6 ± 1.6)		7.4–14.0 × 5.2–13.5 (10.8 ± 1.8 × 9.7 ± 2.1)	
(B)	N.D.[a]	N.D.	7.6–16.2 × 5.4–15.5 (11.8 ± 2.3 × 10.8 ± 2.7)[c]		8.4–13.5 × 8.4–13.5 (11.2 ± 1.8 × 11.2 ± 1.6)[d]	
Sporodochial conidia						
3-septate (D)	27.8–52.9 × 2.5–3.7 (39.4 ± 7.1 × 3.0 ± 0.27)		N.D.	N.D.	N.D.	N.D.
(D/B)	27.3–50.7 × 3.0–3.7 (39.2 ± 6.4 × 3.2 ± 0.19)		34.7–59.0 × 3.0–4.4 (46.4 ± 6.3 × 3.4 ± 0.40)		28.3–59.3 × 3.0–4.2 (44.6 ± 8.6 × 3.5 ± 0.36)	
(B)	31.7–54.4 × 2.7–4.4 (42.8 ± 5.9 × 3.4 ± 0.41)		33.7–61.7 × 3.0–4.2 (46.5 ± 7.7 × 3.4 ± 0.36)		32.0–61.5 × 2.7–4.2 (47.7 ± 7.9 × 3.2 ± 0.47)	
5-septate (D)	43.3–67.4 × 2.7–4.4 (53.6 ± 6.4 × 3.4 ± 0.44)		N.D.	N.D.	N.D.	N.D.
(D/B)	28.0–62.2 × 3.2–3.4 (46.6 ± 9.5 × 3.3 ± 0.12)[e]		47.0–76.0 × 3.0–4.7 (57.9 ± 5.5 × 3.7 ± 0.43)		44.0–69.6 × 3.0–4.2 (56.6 ± 5.3 × 3.5 ± 0.38)	
(B)	47.0–59.0 × 2.5–3.9 (52.4 ± 3.3 × 3.2 ± 0.37)		45.0–69.1 × 2.7–4.7 (59.2 ± 5.3 × 3.5 ± 0.50)		48.2–72.6 × 3.0–4.7 (61.7 ± 5.7 × 3.6 ± 0.50)	

a) N.D.: no conidia produced; b) only 4 conidia; c) 26 conidia; d) 8 conidia; and e) 9 conidia were found and measured.

found in a culture under alternating illumination.

Clavate conidia, which were produced constantly and abundantly by all three strains under all light conditions, were affected by BLB light in their microdimensions. The clavate conidia in MAFF 237512 and 237513 become considerably longer and slightly wider under BLB light than in darkness (Table 2, Figs. 1, 2). MAFF 237511 showed no marked difference in the microdimensions of the conidia under the tested conditions.

Discussion

Microdimensions of the different conidia in the Japanese strains of *F. globosum* agreed very well with the description given by Rheeder et al. (1996), and with the morphology of the ex-type strain deposited at PROMEC, Medical Research Council (MRC 6647), Tygerberg, South Africa. Rheeder et al. (1996) noted infrequent production of pyriform to napiform conidia and considered them as precursor conidia to the globose type. In Japanese strains, smaller pyriform conidia were sometimes produced especially under the BLB light and were morphologically continuous to other globose to subglobose aerial conidia. Rheeder et al. (1996) also commented that the species produced sporodochia by the stimulation of near-UV light. This finding coincides with the reaction of the Japanese strains to BLB illumination. In their morphological and ecophysiological features, such as microdimensions, colony characteristics, mode of conidiogenesis, the absence of chlamydospores, and the production of conidia with/without BLB light, the Japanese strains were found to be identical with those of *F. globosum* in South Africa.

Fusarium globosum was originally isolated from corn kernels harvested in the Butterworth district in South Africa in 1992 (Rheerder et al., 1996), around which a warm temperate to subtropical climate is prevailing. Our Japanese isolates were obtained in 1991 from wheat culms cultivated experimentally on one of the southernmost islands (Ishigaki) in subtropical Japan, quite near Taiwan.

Fusarium globosum has three different types of conidia, i.e., phialidic and holoblastic ones on conidiophores of the aerial mycelium and phialidic ones in sporodochia. Their proportions vary according to light conditions, although some additional factors may be found which will affect the proportional rate of the three conidial types produced. Wicklow (1981) estimated the adaptive value of conidia of microfungi in the ecosystem, and stated that conidia might serve as vehicles by which fungi escape from or survive in unfavorable environments. He also suggested that a fungus that is able to produce one or more conidial types in addition to its sexual stage increases its options for dispersal. Bisby (1943) believed that the conidial habit has greater importance in tropical regions, where a fungus does not have to survive a cold winter, even though it generally has to be prepared for a dry season. Each of the conidial types of *F. globosum* may play its own ecological-biological role, to give the species some advantage in its habitats.

Influences of light on sporogenesis of fungi, including *Fusarium* species, have been studied by different authors (Snyder and Hansen, 1941; Barnett and Lilly, 1950; Aragaki, 1961; Leach, 1965; Trione et al., 1966; Tan, 1974a, b; Honda and Aragaki, 1978a–d; Kilpatrick and Chilvers, 1981; Nirenberg, 1990). Illumination with light of various wavelengths, especially of shorter ranges, such as near-UV, violet to blue light, induced and/or accelerated sporogenesis of many fungal species and strains. Sometimes illumination with light also had a negative effect by inhibiting sporogenesis of some species or strains. A substance, P310, which absorbed UV wavelength of 310 nm was found to be associated with the light-induced sporulation of fungi. It was isolated from different fungal species, such as *Ascochyta pisi* Libert and *Pleospora herbarum* (Pers.:Fr.) Rabenh. (Leach, 1965; Trione et al., 1966). Reactions of many fungi to various light conditions were then described, and morphological alterations of conidia of *Exserohilum rostratum* (Drech.) Leonard & Suggs were intensively studied (Honda and Aragaki, 1978a–d). The mechanisms of photosporogenesis of fungi were speculated on and discussed. Fungal responses to light conditions, however, varied too much depending on species and strains to get a concrete answer to this phenomenon.

The present study describes precisely reactions of Japanese strains of *F. globosum* to BLB light. BLB (near-UV) light induces sporodochial conidiogenesis and suppresses production of the globose conidia in the aerial mycelium. These physiological-morphological features may relate to the ecology of the species and the environmental factors of the habitats. Induction of different types of conidia by different light conditions may also imply the adaptability of the species to its habitats. Production of sporodochial conidia only under BLB light was also reported for *Fusarium sacchari* (Butler) W. Gams (≡*F. sacchari* var. *sacchari*) and *F. phyllophilum* Nirenberg & O'Donnell (=*F. proliferatum* var. *minus* Nirenberg) (Nirenberg, 1976; Gerlach and Nirenberg, 1982; Nirenberg and O'Donnell, 1998). The original hosts and the type localities of both species are on *Saccharum officinarum* L. in India and *Dracaena deremensis* Engl. in Italy, respectively, where the sunshine is more intense than that in regions of higher latitudes. Because of the limited records of *F. globosum*, discussion on morphological and ecological relationships has to wait for future accumulation of data.

For the identification of *Fusarium* species, Nirenberg (1980, 1989, 1990) recommended the application of continuous BLB illumination and complete darkness to culture isolates, as well as the use of a synthetic, low nutritional agar medium, SNA, as the standard method. The combination of both light conditions was essential to obtain data on all the conidial types produced by the Japanese strains of *F. globosum*. In order to be able to follow the splitting species concept of *Fusarium*, precise morphological characterization of the strains to be examined is required, i.e., cultural conditions should be fixed and well controlled, and microdimensions should be

obtained from cultures growing under different standardized conditions to know the variability of the strains. Application of both light conditions to *Fusarium* isolates while growing in culture proved to be quite effective for their morphological and taxonomical analyses. In the case of *F. globosum*, the alternating illumination program of 12 h darkness/12 h BLB light can also be recommended as an abbreviated procedure to observe all three conidial types, because all of them were induced by this light regime. Our observation on the reactions of the strains to the light conditions supports strongly the idea of Nirenberg and may explain its importance.

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