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Host Range of *Aciculosporium take*, the Causal Agent of Witches' Broom of Bamboo Plants

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Abstract The host range of *Aciculosporium take*, the causal agent of witches' broom of bamboos, was surveyed in Japan, and 17 species, 8 varieties, 9 forms and 2 horticultural cultivars in 5 genera of bamboo plants were confirmed as host plants. Of these, 11 species, 6 varieties, 6 forms, and 2 horticultural cultivars in 4 genera were new host plants. Here, in addition to the study of host range, the morphology and characteristics of conidia and conidial germination of the fungus are discussed.

Key words: *Albomyces take*, bamboo taxonomy, conidial morphology, fungi, new host.

Introduction

Bamboo plants with densely ramified young twigs without normal branchlets and normal leaves have recently been observed in many Madake (*Phyllostachys bambusoides* Sieb. et Zucc.) forests in the Kansai area of Japan. This is a typical symptom of witches' broom of Madake. The disease is one of the most destructive causes of the deterioration of bamboo forests and has been well known in Japan since the Meiji era (Hara, 1907; Tsuboi, 1907; Shinohara, 1965). The disease has also been reported in China (Zhu and Huang, 1988) and Taiwan (Chen, 1970) on some *Phyllostachys* bamboo species. The causal fungus, *Aciculosporium take* Miyake (anamorphic stage=*Albomyces take* Miyake), is considered to be an allied species of *Balansia* (Hino, 1962) and therefore treated as a member of the clavicipitaceous fungi (Diel, 1950; Kobayashi, 1992). However, few studies on this fungus and the disease have been reported. Generally, the fungus is believed to attack only bamboo plants belonging to the genus *Phyllostachys* (Kobayashi, 1992), although bamboo species other than *Phyllostachys* have been recorded as host plants in Japan (Hara, 1938). The pathogenicity of the fungus to *Shibataea kumasaca* (Zoll.) Nakai (Okame-zasa) has also been proved by artificial inoculation (Chen, 1970; Shinohara, 1970). Accordingly, the host range of the fungus has not been fully explored. In this study, we describe the host plants of the fungus on the basis of our survey in several bamboo gardens. Furthermore, we investigated some morphological characteristics of the conidia and their germination process of the fungus on artificial culture.

Materials and Methods

The survey of host plants. The study was principally made during 1994–1996 on bamboo forests around Kyoto-shi and the bamboo sample stands in the following botanical gardens: The Kyoto Botanical Garden, Shimogamo Nakaragi-cho, Sakyo-ku, Kyoto-shi; Rakusai Bamboo Park, Ohe Kitafukunishi-cho, Nishikyo-ku, Kyoto-shi; Kamigamo Experimental Forest, Kyoto University, Kamigamo Motoyama, Kita-ku, Kyoto-shi; Kansai Research Center, Forestry and Forest Products Research Institute, Momoyama-cho, Fushimi-ku, Kyoto-shi; Yoro Bamboo Garden in Yoro-Koen, Yoro-cho, Gifu-ken. Other sampling sites from which voucher collections were obtained are indicated as necessary.

Determination of the fungus. Because the anamorphic stage of the fungus was detected throughout the year and the period of the teleomorphic stage is limited to summer, our survey was made on the basis of the anamorphic stage. When the witches' broom symptom was observed, the presence of white spindle-like stromatic pseudoparenchymatal tissues was first confirmed. Then a portion of twigs with symptoms was collected and the presence of conidia or other fungal structures was examined. If conidia were detected, conidial measurements were made on conidia from conidial masses naturally produced on stromatic tissues by fixation with lactophenol. Only the total length was determined because the width was less than 2 μm and precise measurement was difficult.

Observation of some cultural characteristics. The shape of conidia produced on artificial culture was compared with that of naturally produced conidia. Some cultural characteristics were also investigated. In this study, the fungus was cultured on PDA (Difco) and PDB (Difco) media at 25°C in the dark.

Deposition of voucher collections. All of the sample specimens collected in the field were deposited at the National Science Museum, Tokyo with TNS accession numbers TNS F-100,001-100,088. The names of collected bamboo plants with the fungus and their accession numbers are indicated in Tables 1 and 2. Names of the host plants follow Suzuki (1978) or the indication by each bamboo garden.

Results and Discussion

Disease symptoms. The disease symptoms closely matched those reported

Table 1. List of voucher bamboo plants with *Aciculosporium take*.

Japanese common name	Latin name*[1]	Collection site*[2]					
		A	B	C	D	E	F
Ma-dake	*Phyllostachys bambusoides*	—	010	sym	040	055	
Shibo-chiku	var. *marliacea*	001	011	031	—	056	
Ginmei-shibo-chiku	cv. ginmeishibochiku*	—	—	—	—	057	
Kashiro-dake	f. *kashirodake*	—	012	—	041	058	071*a
Kon-shima-dake	f. *subvariegata*	—	—	—	042	—	
Mutsuore-dake	f. *geniculata*	—	013	—	—	—	
Kinmei-chiku	var. *castillonis*	002	014	—	—	059	
Ginmei-chiku	var. *castilloni-inversa*	—	—	—	—	060	072*b
Ougon-chiku	var. *holochrysa*	—	015	—	—	061	
Usan-chiku	cv. usanchiku*	—	—	—	—	062	
Hotei-chiku	*P. aurea*	003	016	032	sym	sym	
Shima-hotei-chiku	f. *albo-variegata*	004	017	—	043	—	
Ginmei-hachiku	var. *flavescens-inversa*	sym	018	—	sym	063	073*a
Ougon-hotei	f. *holochrysa**	—	—	033	—	064	
Kuro-chiku	*P. nigra*	no	no	034	044	no	
Ha-chiku	var. *henonis*	—	no	035	no	no	
Unmon-chiku	f. *boryana*	no	no	036	no	no	
Shima-hachiku	f. *albo-variegata*	—	019	—	045	—	
Tosa-torafu-dake	var. *tosaensis*	—	020	no	no	065	
Meguro-chiku	f. *meguorchiku*	no	no	sym	no	066	
Taiwan-ma-dake	*P. makinoi**	no	021	no	no	no	
Inyo-chiku	*P. tranquillans*	no	022	—	046	067	
Hakuho-kanchiku	*P. dulcis**	—	023	—	047	no	
Hime-hachiku	*P. humiris**	no	no	—	048	no	
Okame-zasa	*Shibataea kumasaca*	005	024	037	no	068	
Narihira-dake	*Semiarundinaria fastuosa*	006	025	038	049	069	
Ao-narihira	var. *viridis*	no	026	—	050	no	
Nikko-narihira	*S. yoshi-matsumurae*	007	027	—	051	—	
Bizen-narihira	*S. okuboi*	008	028	—	052	070	
Kenashi-Yasha-dake	*S. tatebeana*	—	029	—	053	—	
Rikuchu-dake	*S. kagamiana*	—	030	—	054	no	
Taimin-chiku	*Pleioblastus gramineus*	009	no	—	no	no	
Ne-zasa	*Pleioblastus* Sect. *Nezasa* sp.	no	no	no	no	no	074*c
Chimaki-zasa	*Sasa* Sect. *Sasa* sp.	no	no	no	no	no	075*d
Nambu-suzu	*Sasa* Sect. *Lasioderma* sp.	no	no	039	no	no	

*[1] The bamboo names are after Suzuki (1978): Index to Japanese Bambusaceae. The names with (*) are followed by the indication of each bamboo garden.

*[2] Collection sites: A, Kansai Research Center, Forestry & Forest Products Research Institute; B, The Kyoto Botanical Garden; C, Yoro Bamboo Garden; D, Kamigamo Experimental Forest, Kyoto University; E, Rakusai Bamboo Park; F, Others (*a, Takeda Pharmaceutical Plant Garden at Kyoto-shi; *b, The Fukiage Gardens in the Imperial Palace Grounds; *c, Kohata, Uji-shi, Kyoto-fu, same as 079 in the Table 2; *d, Kyoto University Forest in Ashiu).

—, Not planted; no, Not confirmed; sym, Symptom was confirmed.

Table 2. List of vouchers of *P. bambusoides* with *Aciculosporium take* other than those in Table 1.

076; Isonokami, Tenri-shi, Nara-ken	083; Daigo, Fushimi-ku, Kyoto-fu
077; Isonokami, Tenri-shi, Nara-ken	084; Rokujizo, Uji-shi, Kyoto-fu
078; Sumiyama, Uji-shi, Kyoto-fu	085; Bicchu-Matsuyama, Okayama-ken
079; Kohata Kanakusahara, Uji-shi, Kyoto-fu	086; Shizu, Sakura-shi, Chiba-ken
080; Kohata Kanakusahara, Uji-shi, Kyoto-fu	087: Sakura, Sakura-shi, Chiba-ken
081; Shirakawa, Uji-shi, Kyoto-fu	088; Amakubo, Tsukuba, Ibaraki-ken
082; Shirakawa, Uji-shi, Kyoto-fu	

in the literature (Tsuboi, 1907; Miyake, 1908; Hara, 1938; Hino and Katsumoto, 1961; Shinohara, 1965). Namely, the bamboo twigs attacked by the fungus were repeatedly ramified with deformations. Plants often drooped from the canopy and gave a dirty brownish impression due to poor leaf growth. When the fungus attacked ornamental bamboo plants, the beauty of the bamboo stand was spoiled by the drooping of brownish-parts diseased by witches' broom. This spoilage was widely distributed in bamboo culms at the margins of Madake forests near Kyoto and other areas of the Japanese archipelago. The diseased parts gradually defoliated and the whole plant died, probably because loss of leaves led to decreased photosynthesis. Production of bamboo culms and bamboo sprout are also decreased (Tsuboi, 1907). New culms with elongated or shortened nodes and with irregularly proliferated branches occasionally emerged from aerial parts of underground rhizomes. These symptoms were recognized on *Semiarundinaria fastuosa* (Mitf.) Makino (Narihira-dake) (Fig. 3), *Pleioblastus gramineus* (Bean) Nakai (Taimin-chiku) (Fig. 1), and *Sasa* Sect. *Lasioderma* sp. (Nambu-suzu) (Fig. 4), respectively.

Host range of the fungus. Conidial sizes were similar among the different host bamboo plants examined, falling within the range of variation of the specimens on *P. bambusoides* collected from stands in different localities (Tables 3, 4). This indicates that the causal agent of witches' broom of these bamboo plants is one species, the *Albomyces* stage of *Aciculosporium take.*

In the field, we found the disease mainly on *P. bambusoides* stands. In a rare case, the disease was recognized on *Pleioblastus* Sect. *Nezasa* sp. (Ne-zasa) (Fig. 4) growing under heavily diseased Madake-forest. We also detected the disease on *Sasa* Sect. *Sasa* sp. (Chimaki-zasa) (Fig. 5) at the Kyoto University Forest in Ashiu, in Miyama-cho, Kyoto-fu. In this case, the host plant is formed a pure stand with no other bamboo plants around it. Then we surveyed the disease in several bamboo gardens. As a result, the bamboo plants listed in Table 1 were confirmed as host plants of the fungus. The results of conidial measurements of the fungus from the above listed host plants are given in Table 3.

In the literature, few species are recorded as the host plants of the fungus. In

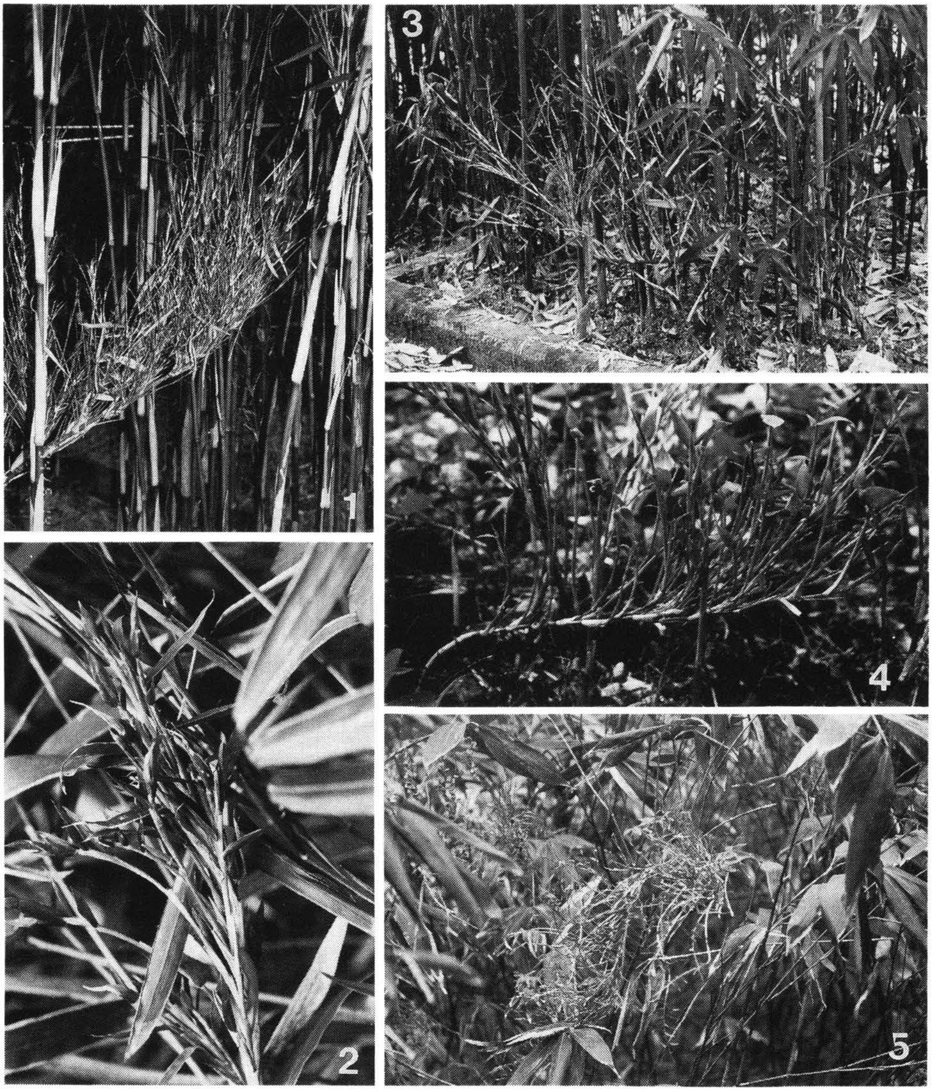

Fig. 1. The disease symptom on the new twigs of *Pleioblastus gramineus* (Taimin-chiku) grown from the aerial part of underground rhizomes.

Fig. 2. The disease symptom on *Pleioblastus* Sect. *Nezasa* sp. (Ne-zasa).

Fig. 3. The disease symptom on new twigs of *Semiarundinaria fastuosa* (Narihira-dake) grown from the aerial part of underground rhizomes.

Fig. 4. The disease symptom on the new twigs of *Sasa* Sect. *Lasioderma* sp. (Nambu-suzu) grown from the aerial part of underground rhizomes.

Fig. 5. The disease symptom on *Sasa* Sect. *Sasa* sp. (Chimaki-zasa).

Table 3. Conidial lengths of *Aciculosporium take* from different bamboo plants.

Bamboo species	Collection site					
	A	B	C	D	E	F
Phyllostachys bambusoides	—	48.0±4.1	—	46.2±3.7	47.7±3.2	
var. *marliacea*	44.6±5.6	48.2±3.5	44.3±7.2	—	45.8±3.8	
cv. ginmeishibochiku	—	—	—	—	47.3±4.6	
f. *kashirodake*	—	48.8±3.9	—	48.2±4.3	47.1±3.6	50.1±3.9*a
f. *subvariegata*	—	—	—	42.5±3.7	—	
f. *geniculata*	—	49.2±3.5	—	—	—	
var. *castillonis*	44.6±4.4	46.2±3.5	—	—	47.5±3.0	
var. *castilloni-inversa*	—	—	—	—	48.0±3.8	48.2±3.7*b
var. *holochrysa*	—	45.5±3.6	—	—	47.3±4.1	
cv. usanchiku	—	—	—	—	47.0±3.1	
P. aurea	49.6±2.9	46.4±4.4	45.6±4.6	—	—	
f. *albo-variegata*	46.8±4.7	47.3±4.5	—	47.7±3.4	—	
var. *flavescens-inversa*	—	45.1±3.6	—	—	48.0±3.8	48.4±4.3*a
f. *holochrysa*	—	—	44.7±5.3	—	48.7±3.4	
P. nigra	—	—	45.0±3.4	47.8±3.2	—	
var. *henosis*	—	—	46.6±4.0	—		
f. *boryana*	—	—	43.2±3.6	—	—	
f. *albo-variegata*	—	49.4±5.1	—	46.8±2.8	—	
var. *tosaensis*	—	46.5±4.2	—	—	46.8±3.5	
f. *megurochiku*	—	—	—	—	44.4±3.5	
P. makinoi	—	48.4±4.1	—	—	—	
P. tranquillans	—	48.5±3.7	—	44.6±4.1	47.8±3.4	
P. dulcis	—	46.6±3.9	—	47.0±3.5	—	
P. humiris	—	—	—	46.6±3.5	—	
Shibataea kumasaca	48.5±3.9	47.2±3.0	48.1±3.4	—	47.2±3.4	
Semiarundinaria fastuosa	43.6±4.1	41.8±3.9	43.6±4.3	45.9±3.4	46.5±4.0	
var. *viridis*	—	44.9±3.4	—	39.8±4.1	—	
S. yoshi-matsumurae	42.7±5.5	44.5±3.9	—	45.5±2.6	—	
S. okuboi	45.0±4.4	48.8±3.2	—	46.3±3.5	48.5±3.7	
S. tatebeana	—	48.4±5.2	—	48.6±3.7	—	
S. kagamiana	—	46.0±4.1	—	45.6±3.1	—	
Pleioblastus gramineus	41.9±4.4	—	—	—	—	
Sect. *Nezasa* sp.	—	—	—	—	—	43.0±3.9*c
Sasa Sect. *Sasa* sp.	—	—	—	—	—	42.7±2.8*d
Sect. *Lasioderma* sp.	—	—	48.3±3.8	—	—	

The bamboo names and collection sites are the same as in Table 1.
Conidial lengths (in μm) are recorded as average value±S.D. (n=50).

this study, however, the fungus was recognized on 17 species, 8 varieties, 9 forms and 2 horticultural cultivars in 5 genera of bamboo plants in the bamboo gardens surveyed. Consequently, we can add 11 species, 6 varieties, 6 forms, and 2 horticultural cultivars in 4 bamboo genera as new host plants. The newly discovered host plants are: *Phyllostachys bambusoides* cv. ginmeishibochiku (Gin-

Table 4. Conidial lengths of *Aciculosporium take* on *Phyllostachys bambusoides* collected from differnt locations.

Voucher no.	Conidial length	Voucher No.	Conidial length
010 (B)	48.0±4.1	081 (Shirakawa)	43.3±4.6
040 (C)	46.2±3.7	082 (Shirakawa)	44.3±3.4
055 (E)	47.7±3.2	083 (Daigo)	44.8±3.7
076 (Isonokami)	40.5±3.2	084 (Rokujizo)	45.7±3.6
077 (Isonokami)	48.5±3.7	085 (Takahashi)	44.6±3.1
078 (Sumiyama)	50.2±3.9	086 (Shizu)	44.4±4.3
079 (Kohata)	43.5±3.2	087 (Sakura)	42.7±4.7
080 (Kohata)	46.0±3.3	088 (Amakubo)	46.4±4.7

Locations are indicated in Tables 1 and 2.
Conidial lengths (in μm) are recorded as average value±S.D. (n=50).

mei-shibo-chiku), *P. bambusoides* f. *subvariegata* Makino ex Tsuboi (Kon-shima-dake), *P. bambusoides* f. *geniculata* (Nakai) Muroi (Mutsuore-dake), *P. bambusoides* var. *castillonis* (Marliac ex Carr.) Makino (Kinmei-chiku), *P. bambusoides* var. *castilloni-inversa* Houz. de Leh. (Ginmei-chiku), *P. bambusoides* var. *holochrysa* Pfitzer ex Houz. de Leh. (Ougon-chiku), *P. bambusoides* cv. usanchiku (Usan-chiku), *P. aurea* f. *albo-variegata* Makino (Shima-hachiku), *P. aurea* var. *flavescens-inversa* (Houz. de Leh.) Nakai (Ginmei-hachiku), *P. aurea* f. *holochrysa* (Ougon-hotei), *P. nigra* var. *tosaensis* Makino ex Tsuboi (Tosa-torafu-dake), *P. nigra* f. *megurochiku* (Makino) Nakai (Meguro-chiku), *P. tranquillans* (Koidz.) Muroi (Inyo-chiku), *P. dulcis* McClure (Hakuho-kanchiku), *P. humiris* Muroi (Hime-hachiku), *Semiarundinaria fastuosa* var. *viridis* Makino (Ao-narihira), *S. yoshi-matsumurae* Muroi (Nikko-narihira), *S. okuboi* Makino (Bizen-narihira), *S. tatebeana* Muroi (Kenashi-yashadake), *S. kagamiana* Makino (Rikuchu-dake), *Pleioblastus gramineus* (Bean) Nakai (Taimin-chiku), *Pleioblastus* Sect. *Nezasa* sp. (Ne-zasa), *Sasa* Sect. *Sasa* sp. (Chimaki-zasa), and *Sasa* Sect. *Lasioderma* sp. (Nambu-suzu). We also confirmed the natural infection on *Sibataea kumasaca* (Okame-zasa), which had been reported as a host plant of the fungus by artificial inoculation (Chen, 1970; Shinohara, 1970). From the results of this study and the list of Hara (1936), it is clear that some *Phyllostachys* species and their horticultural cultivars, and the members of the genus *Semiarudinaria* were the most frequently attacked by the fungus. However, Moso-chiku (*Phyllostachys pubescens* Mazel ex Houz.) and its horticultural cultivars, which are widely cultivated in Japanese archipelago, especially in the suburbs of Kyoto-shi, was free of the fungus in our survey. This plant has been reported as a host of the fungus in Taiwan (Chen, 1970). This might be due to a difference in susceptibility of the plant depending on the geographical location or a problem in identification of Moso bamboo. In the genus *Semiarudinaria*, we could not find the fungus on *S. yashadake* (Makino) Makino (Yasha-dake). This

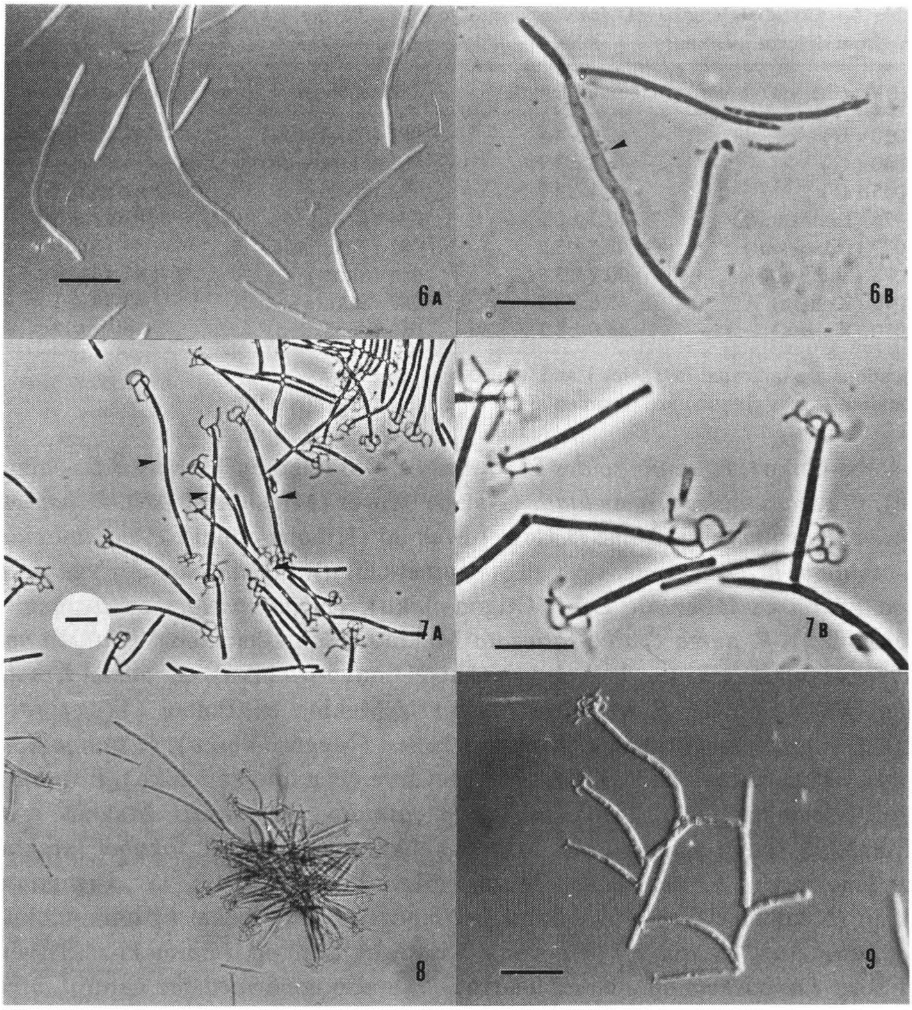

Fig. 6. Naturally produced conidia of *Aciculosporium take*. A. Conidia. B. Conidium with middle septum (arrow head).

Fig. 7. Cultural type conidia produced by germination of natural conidia of *Aciculosporium take*. A. Cultural type conidial mass with end-to-end germination on the agar medium (arrow heads). B. Germination from basal part (arrow head).

Fig. 8. Mycelium colony of *Aciculosporium take* on the agar medium.

Fig. 9. Mycelium with cultural type conidia of *Aciculosporium take* on the agar medium. (Bars indicate 10 μm.)

may also reflect a problem in host taxonomy. These results were interesting and may open the way to phylogenetical consideration of these species on the basis of affinity with the fungus.

Difference in conidial morphology in nature and in artificial culture. The conidia from field collections had the same morphological characteristics as already reported (Fig. 6). When we tried to isolate the fungus monoconidially on agar medium, it was difficult to establish cultural isolates. Then we tried to isolate the fungus by mass inoculation of stromatic tissues on agar medium. Slow-growing pustules appeared on the agar medium and almost all colonies stopped growing at this stages (Fig. 8). Mycelial growth was very rare and occurred by chance. The reasons for this phenomenon were revealed by microscopic observations. The naturally formed conidia of the fungus germinated from both ends and formed two dichotomously branched appendages at their apex (Fig. 7), which have been referred to as germinating hyphae (Shinohara, 1965, 1966). The appendages, however, seldom produced hyphae during our observations. Consequently, the germinated conidia stopped growing at this end-to-end stage (Fig. 7). When they separated by breaking at the middle septum, new germination occurred from the basal end and formed the same structures with apical appendages as Fig. 7. This kind of structure was also produced from the hyphae as a lateral branch as shown in Fig. 9. We conclude that this structure is one kind of conidium. Next we tried to culture the fungus in liquid medium by shaking. Growth proceeded by repeated separation at the bases of these conidia, and many single conidia or unseparated few-celled conidia with appendages were observed in the culture. Thus the fungus has two types of conidia: naturally produced filiform conidia with swollen ends and one septum at the middle (Fig. 6), and cultural type conidia with apical appendages (Fig. 7). These cultural type conidia with appendages might function for dispersal in the field by water droplets on the bamboos, like those of amphibious fungi in streams (Kendrick, 1992).

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