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FULL PAPER

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Exobasidium symploci-japonicae var. *carpogenum* var. nov. causing Exobasidium fruit deformation on *Symplocos lucida* in Japan

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Abstract *Exobasidium symploci-japonicae* var. *carpogenum*, causing Exobasidium fruit deformation on *Symplocos lucida* collected in Fukuoka Prefecture, Japan, is newly described based on morphological observations of hymenial structure and mode of basidiospore germination. This new variety differs morphologically from the type variety, particularly in the septal number of basidiospores and in the shapes and sizes of conidia formed on the medium. Colonies of this new variety are also distinguishable from those of the type variety by yeast-like growth, morphology, and color of colonies.

Key words Basidiomycetes · Culture · *Exobasidium* · Fruit deformation · Japan

Introduction

Recently, fruit deformation of *Symplocos lucida* Siebold et Zucc. and *S. myrtacea* Siebold et Zucc. by *Exobasidium* species was reported in Fukuoka Prefecture, but the species of these causal fungi have not been determined (Ogawa 1996, 2000). Fruit deformation has not been reported on *Symplocos* spp. in Japan. Two species of *Exobasidium* have been recorded on *Symplocos* spp. in Japan (Ezuka 1991; Hirata 1981; Kusano 1907). *Exobasidium symploci-japonicae* Kusano et Tokubuchi invades shoots and undeveloped young leaves of *S. lucida* and causes Exobasidium leaf blight (Ezuka 1991; Kusano 1907). *Exobasidium sakataniense* S. Hirata invades shoots and undeveloped young leaves of *S. theophrastaefolia* Siebold et Zucc. and causes typical Exobasidium leaf blight and also small leaf spot on developing young leaves. This species is distinguished from *E. symploci-japonicae* in the number of sterigmata and size of basidiospores (Hirata 1981).

In 2001, we were able to obtain fresh specimens of fruit deformation of *S. lucida* in Fukuoka Prefecture and observe their morphology. We propose a new variety of *E. symploci-japonicae* based on comparative morphology with *Exobasidium* species on *Symplocos* spp.

Materials and methods

Morphological observations

For light microscopy, materials were prepared as described previously (Nagao et al. 2003). Observations and measurements of conidia were conducted from the pure culture isolates on Difco potato dextrose agar (PDA). For scanning electron microscopy (SEM), samples were prepared and observed as described previously (Nagao et al. 2001). Fresh materials of Exobasidium fruit deformation and leaf blight on *S. lucida* collected in the field were used for morphological observations. Materials were deposited in the Mycological Herbarium of Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH), and the Herbarium of the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (NIAES). Material of Exobasidium fruit deformation (TSH-B0090) was collected in Kuroyama, Okagaki-machi, Onga-gun, Fukuoka Prefecture, June 22, 2001, by S. Ogawa, where the culture was obtained and deposited

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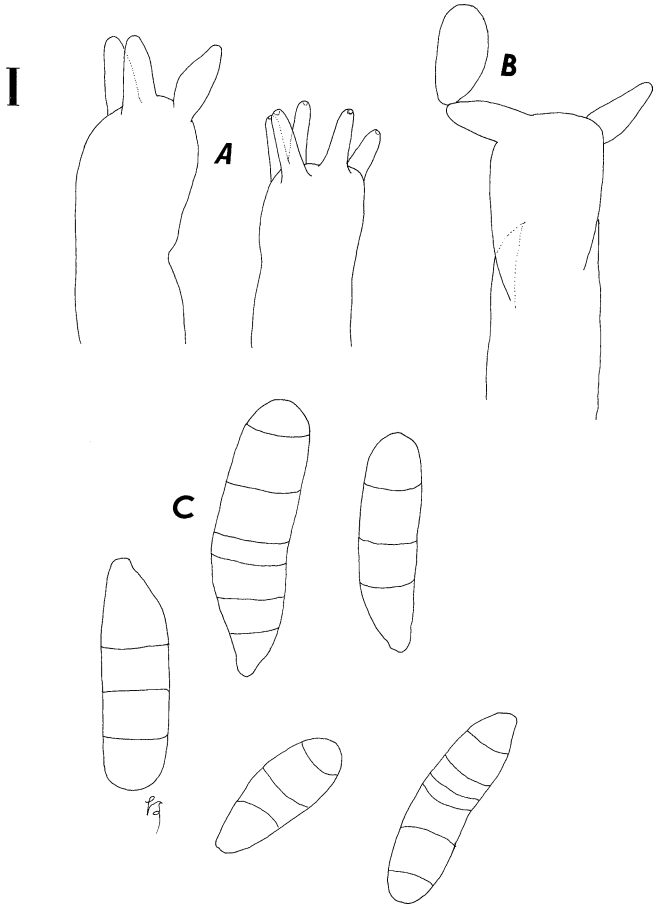

Fig. 1. Basidia and basidiospores of *Exobasidium symploci-japonicae* var. *carpogenum* formed on the infected fruit on *Symplocos lucida*. Basidia (**A**), basidium with immature basidiospore (**B**), and basidiospores (**C**) were collected in Fukuoka Prefecture (TSH-B 0090). *Bar* 3 μm

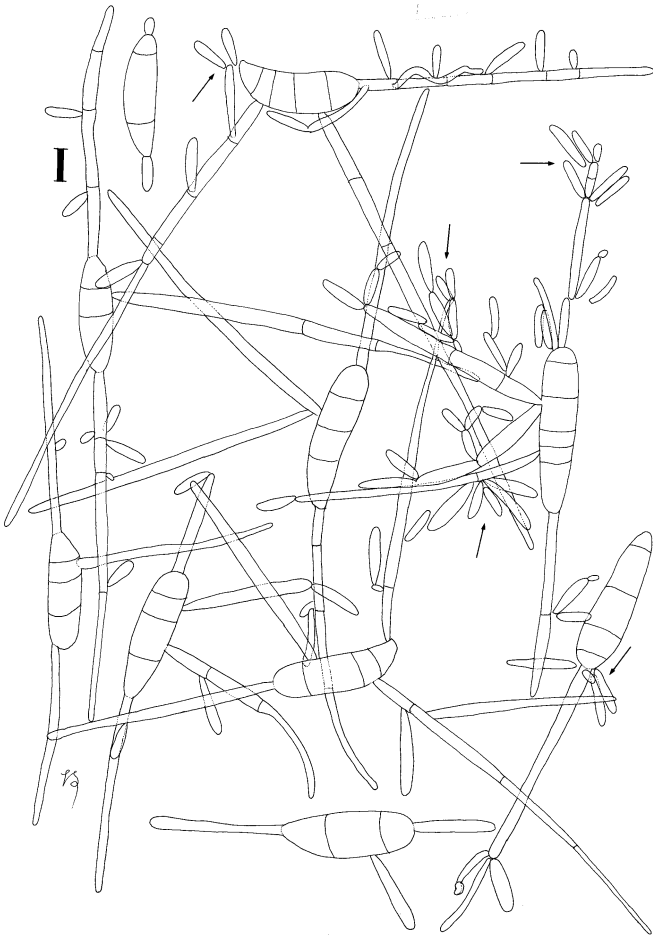

Fig. 2. Basidiospore germination of *E. symploci-japonicae* var. *carpogenum* (TSH-B 0090) on potato dextrose agar (PDA) after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 5 μm

as MAFF238620. For comparison, three samples of *E. symploci-japonicae* var. *symploci-japonicae* were collected: TSH-B0040 in Shinji-machi, Ohara-gun, Shimane Prefecture, June 22, 2000, by T. Kobayashi; NIAES 20520 in Kuroiwa, Okagaki-machi, Onga-gun, Fukuoka Prefecture, June 2002, by J. Onagamitsu; and NIAES 20521 in Katuki-Shimin-no-mori, Chiyo, Yahata-Nishi-ku, Kitakyushu-shi, Fukuoka Prefecture, June 16, 2002, by Y. Usami. Cultures were obtained and deposited as MAFF238605, 238810, and 238811, respectively. Several herbarial materials were examined for comparative morphology; six specimens on *S. lucida* in the Herbarium of the Hokkaido University Museum (SAPA), two specimens on *S. lucida*, and four specimens on *S. theophrastaefolia* collected by Prof. Hirata in the Herbarium of Agricultural Museum, Faculty of Agriculture, Miyazaki University.

Culture of basidiospore isolate

Fresh infected materials were kept in a plastic bag for vegetables until newly sporulating lesions were observed. Colonies from a single basidiospore were obtained from the fresh materials as described previously (Nagao et al. 2003). Cultures were kept in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, and also deposited in National Institute of Agrobiological Sciences (MAFF). An additional culture of *E. symploci-japonicae* var. *symploci-japonicae* IFO7790 was also examined.

Taxonomy

Exobasidium symploci-japonicae Kusano et Tokubuchi var. ***carpogenum*** Nagao et S. Ogawa, var. nov.

Figs. 1, 2

Fructus infecti superficie viridiflavi vel viridescentes, hypertrophici. A typo differt basidiosporis 3–6-septatis. Conidia hyalina, continua, laevia, linearia, globosa, ovata, lacrimiformia, subfusiformia vel claviformia, 2–9 × 1–2 μm. Coloniae in PDA restricte crescentes, post 21 dies maxime 16 mm diam. attingens, ad ambitum irregulares, corrugatae, glutinosae, ex hyphis circa 1 μm latis et conidiis constantes,

persicinae, in agaro non pigmentiferae; reversum pallide persicinum.

Holotypus in fructibus *Symploci lucidae* Siebold et Zucc., Kuroyama, Okagaki-machi, Onga-gun, Fukuoka Prefecture in Japonia, 22 VI 2001, S. Ogawa leg., in Herbario Instituti Agriculturae et silviculturae Universitatis Tsukubensis, Tsukuba, Japonia (TSH-B 0090) conservatus.

Isotypus in Herbario Instituti Nationalis Scientiae Agro-Environmentalis, Tsukuba, Japonia conservatus (NIAES 20530).

Etymology: The Latin word *carpogenus* refers to the infecting part of *S. lucida*.

The hymenium was composed of basidia with 2–5 sterigmata and conidia. Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 8–68 × 6–9 μm (Fig. 1A,B). Basidia emerged directly from the host surface or through stomata. Basidia were not fasciculate. Sterigmata were 1.5–2.5 μm in diameter at the base and 4–6 μm in height, emerging outwardly and tapering toward the tip (Fig. 1A,B). Basidiospores were ellipsoid to ovoid, 13–23 × 4–6 μm, hyaline, smooth, one-celled when formed, becoming septate with 3–6 septa (Fig. 1C). Septate basidiospores germinated after 6 h when dropped on the agar surface. Germ tubes of the basidiospores emerged from each cell and produced conidia at the tip of germ tubes 12 h after dropping (Fig. 2). Hyphae grew into pseudohyphae and branched. Conidia were spherical, oval, lacrimiform, subfusiform, and clavulate, 2–9 × 1–2 μm, and budded or germinated polarly (Fig. 4A). Conidia budded to produce daughter cells polarly and also developed germ tube or hyphae. Colonies on PDA grew gradually, to a maximum 16-mm diameter in 21-day incubation, and were wrinkled irregularly around the periphery. The surface of the colonies was pink to pale pink and corrugate. Colonies were glutinous and did not fix on the agar surface. Colonies were composed of partially elongated pseudohyphae and conidia. Conidial formation did not produce a powdery appearance. The reverse of colonies was also pale pink. Dark pigment was not produced on PDA (Fig. 6A). Colonies from conidia showed the same morphological features as those from basidiospores.

Genus *Exobasidium* is characterized by the clavate to cylindrical basidia with erumpent sterigmata and ellipsoidal basidiospores, and there is no remarkable ornamentation on the surface of basidium and basidiospore. Its host range is wide, and the symptoms are galls on leaves, buds, flowers, fruit, and even on trunk, leaf blister, leaf blast, shoestring leaf, and witches' bloom. The taxonomy of *Exobasidium* has been argued (Burt 1915; Ezuka 1991; McNabb 1962; Nannfeldt 1981; Savile 1959; Sundström 1964). These arguments are attributed to the simple morphology of taxonomic characters and the variable symptoms and wide host range of this genus. We followed Nannfeldt's species concept and compared the morphology of basidia, basidiospores, and sterigmata and the mode of basidiospore germination.

Among 101 taxa of *Exobasidium* having been validly described so far, the following 2 species show some morphological similarities to the present taxon. *Exobasidium sakataniense* differed from *E. symploci-japonicae* var. *carpogenum* in number of sterigmata and size of basidiospores. Numbers of sterigmata of *E. symploci-japonicae* var. *carpogenum* were two to five, whereas *E. sakataniense* had two. Length of basidiospores of var. *carpogenum* ranged smaller than those of *E. sakataniense. Exobasidium symploci-japonicae* var. *symploci-japonicae* differed from *E. symploci-japonicae* var. *carpogenum* in the septal number of basidiospores. Basidiospores of *E. symploci-japonicae* var. *symploci-japonicae* have dominantly 0–4 septa at maturity (Table 1) (Ezuka 1991). Basidiospores of this new variety have 3–6 septa. The septal number of basidiospores overlapped, at about 3–4. Modes of septal number of the basidiospores varied with the two varieties. Variety *symploci-japonicae* deviated to less than 3-septated basidiospores, whereas the new variety had more than 3-septated ones (Fig. 9). Width of basidia, sizes of sterigmata, and sizes of basidiospores of this new variety were smaller than those of *E. symploci-japonicae* var. *symploci-japonicae*, but overlapped in the lower ranges of those of *E. symploci-japonicae* var. *symploci-japonicae* (Table 1). Numbers of sterigmata were the same in both varieties. Basidiospore germination of both varieties was of mycelial form.

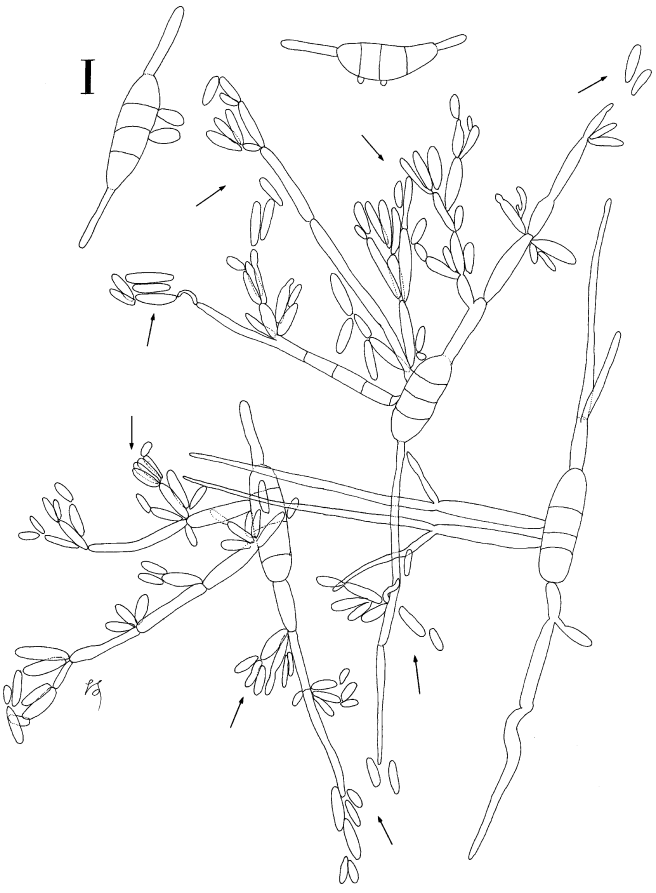

Fig. 3. Basidiospore germination of *E. symploci-japonicae* var. *symploci-japonicae* (TSH-B 0040) on PDA after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 5 μm

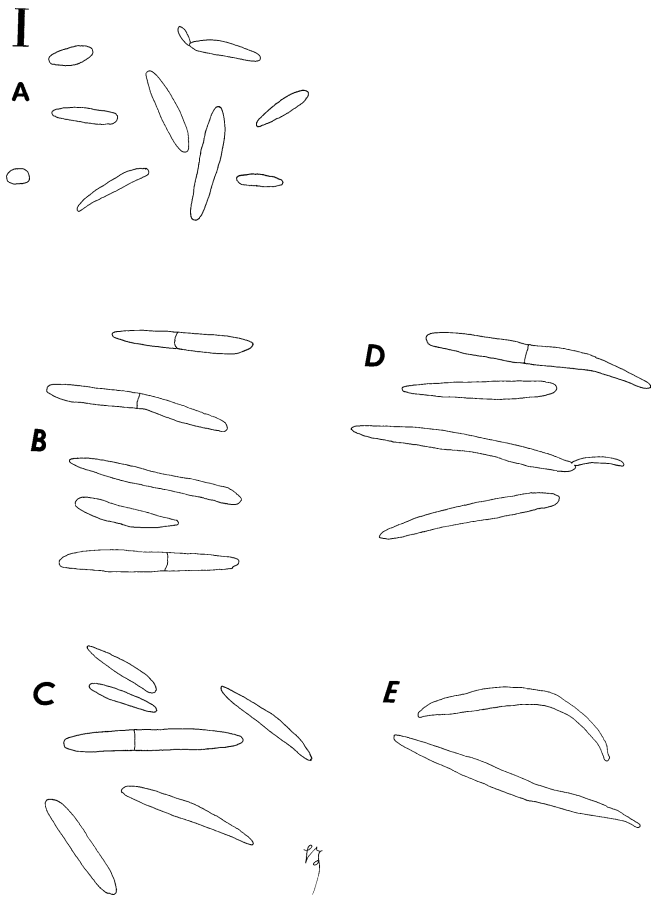

Fig. 4. Conidia of *E. symploci-japonicae* var. *carpogenum* MAFF 238620 (**A**) and var. *symploci-japonicae* MAFF 238605 (**B**), IFO7790 (**C**), MAFF 238810 (**D**), and MAFF 238811 (**E**) produced on PDA in 21-day incubations at 22°C. *Bar* 3 μm

The morphology of *Exobasidium* sp. on *S. myrtacea* has been briefly reported (Ogawa 2000). Size of basidiospores on *S. myrtacea* was in the same range as *E. symploci-japonicae* var. *carpogenum*, but septal number of basidiospores on *S. myrtacea* was two. Number of sterigmata was not determined. Although we could not precisely compare the morphology of *E. symploci-japonicae* var. *carpogenum* with *Exobasidium* sp. on *S. myrtacea*, the septal numbers of basidiospores of these two fungi were different. We considered that these morphological features rank this fungus as a new variety of *E. symploci-japonicae.*

In examining the herbarium materials, no fruit deformation was seen on *S. lucida* and on *S. theophrastaefolia.* All specimens showed the symptoms on shoots and undeveloped young leaves. Basidiospores and basidia could scarcely be detected on these materials.

Conidial shapes and sizes of the new variety markedly differed from those of *E. symploci-japonicae* var. *symploci-japonicae*. Conidia of *E. symploci-japonicae* var. *symploci-japonicae* (IFO7790, MAFF 238605, MAFF 238810, and MAFF 238811) were 7–23 × 1–2 μm, but those of the new variety (MAFF 238620) were 2–9 × 1–2 μm (Table 1). Ezuka (1991) reported conidial sizes of var. *symploci-*

Table 1. Morphological characteristics of *Exobasidium* species on *Symplocos* spp.

Taxon	Size of basidia (μm)	Size of sterigmata (μm)	Number of sterigmata	Size of basidiospores (μm)	Number of septa of basidiospores	Size of conidia (μm)	Number of septa of conidia	References
E. sakataniense	33–55 × 5–8	5–9 × 3–4	2	19–28 × 4–6	(2)3	7–18 × 1.5–2	0	Trans Mycol Soc Jpn 22 (1981):61–63
E. symploci-japonicae var. *carpogenum* TSH-B 0090	8–68 × 6–9	4–6 × 1.5–2.5	2–5	13–23 × 4–6	3–6	2–9 × 1–2	0	
E. symploci-japonicae var. *symploci-japonicae*	120–140 long	nd	(2)4(5)	17–22 × 6–7	nd	nd	nd	Bot Mag Tokyo 21 (1907):138–139
NIAES 10525	100–150 × 8–13	5–8 × 2–3	(2–)3–4(–5)	18–25(–27) × 5–6.5	(1–)3	10–27 × 2–3	0	Trans Mycol Soc Jpn 32 (1991):169–185
IFO7790	nd	nd	nd	nd	nd	(6)7–23 × 1–1.5	0–1	
TSH-B 0040	nd	nd	nd	nd	nd	11–21 × 1–1.5	0–1	
NIAES 20520	33–66 × 5–8	4–7 × 1.5–3	2–4	15–22 × 4.5–7	0–4(6)	(14)16–22(32) × 1.5–2	0–1	
NIAES 20521	22–46 × 7–9	4–7 × 1.5–2.5	2–3	13–23 × 4–7	0–4	13–22 × 1–2	0–1	

nd, not determined

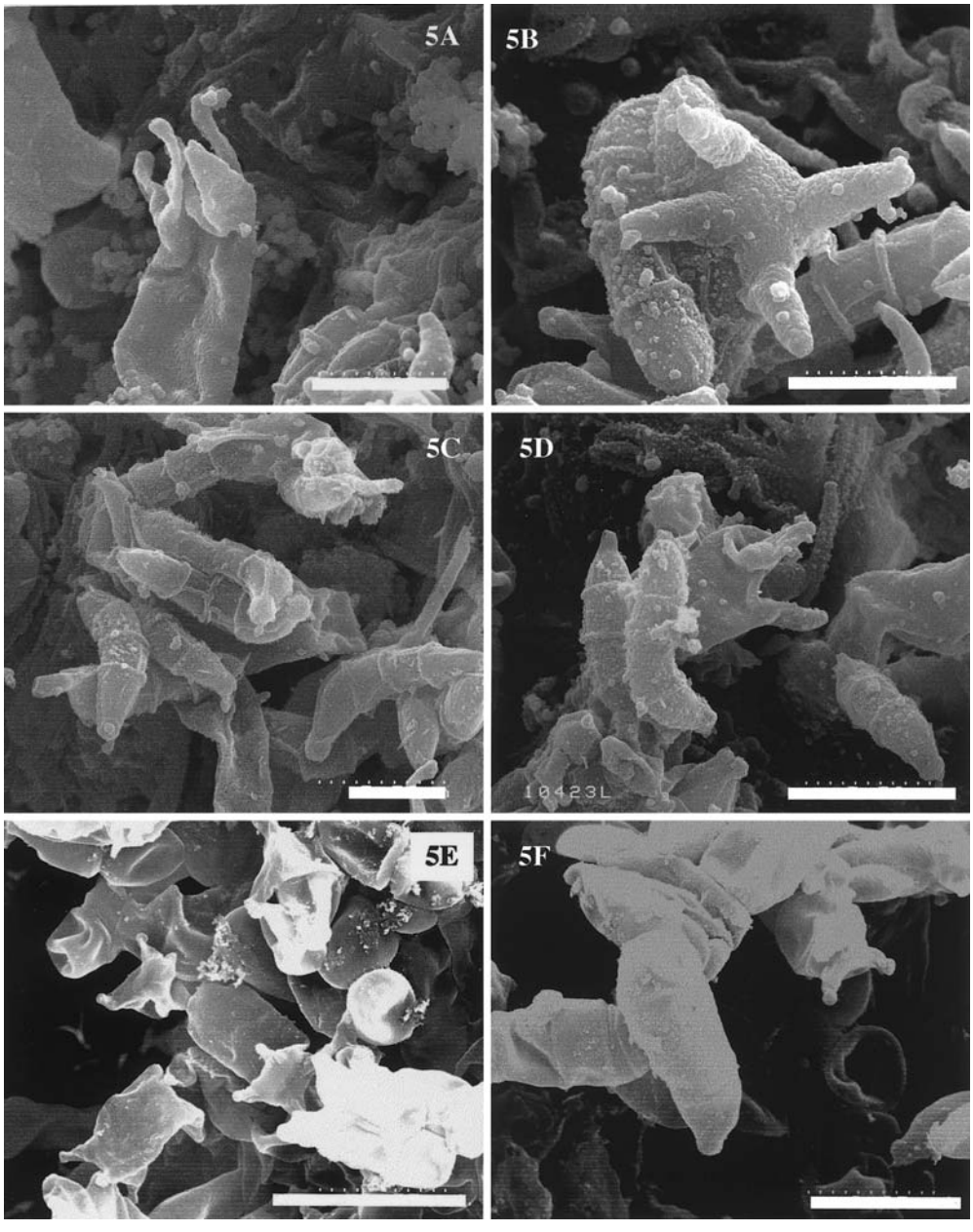

Fig. 5. Hymenium of *E. symploci-japonicae* var. *carpogenum* and var. *symploci-japonicae* observed by scanning election microscopy (SEM). **A** Basidium of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **B**, **D** Basidium and basidiospores of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **C** Basidiospores of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **E** Basidia of *E. symploci-japonicae* var. *symploci-japonicae* on infected leaf of *S. lucida*. Two to four sterigmata were seen. **F** Basidium and basidiospores of *E. symploci-japonicae* var. *symploci-japonicae*. *Bars* 6.5 μm

japonicae (NIAES 10525) as 10–27 × 2–3 μm without septum. These spore sizes were larger and wider than those of our observations. Conidia of the new variety were spherical, oval, lacrimiform, subfusiform, and clavulate (Fig. 4A), whereas those of *E. symploci-japonicae* var. *symploci-japonicae* were long fusiform to linear, sometimes being bent (Fig. 4B–E; Ezuka 1991), and its hyphae inclined to elongate rather than branching. Both germinations started from the tips of conidia. Although germination of basidiospores of both varieties was achieved with the mycelial form, the way of conidial germination was specific to the varieties. Conidial germinations of the new variety were both conidial and mycelial in form, but those of *E. symploci-japonicae* var. *symploci-japonicae* were mycelial in form.

Characteristics of colonies of both varieties were also remarkable. Colonies of *E. symploci-japonicae* var. *symploci-japonicae* composed of intricate pseudohyphae grew radially on PDA, to a maximum 33 mm diameter in 21 days incubation, and fixed on the agar surface (Fig. 6B–E), whereas colonies of the new variety composed of partially elongated pseudohyphae and conidia showed yeast-like growth and were wrinkled irregularly around the periphery (Fig. 6A). Anamorphic characteristics were remarkably different between the two varieties. Sundström (1964) demonstrated that the cultural characteristics (color, colony margination, texture, and mode of growth) and physiological activities led to grouping the strains into host races. Later, Nannfeldt (1981) proposed nine species based on the nine host races named by Sundström, which were classified into two species, *myrtilli* and *vaccinii*. In the case of var. *carpogenum*, the morphology of the teleomorph was not different enough to establish the new species.

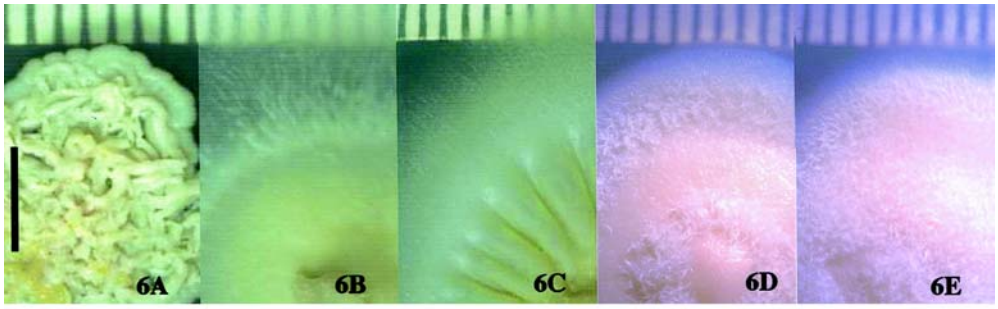

Fig. 6. Morphology and coloration on PDA. Surface of colonies of *E. symploci-japonicae* var. *carpogenum* MAFF 238620 (**A**). Surface of colonies of *E. symploci-japonicae* var. *symploci-japonicae* MAFF 238605 (**B**), IFO7790 (**C**), MAFF 238810 (**D**), and MAFF 238811 (**E**), respectively. *Bar* 5 mm

Fig. 7. Symptoms on Exobasidium fruit deformation on *S. lucida* by *E. symploci-japonicae* var. *carpogenum*. **A** Fruit deformation covered with white hymenia observed on June 2001 in Fukuoka Prefecture. **B** Appearance of fruit deformation (*left* and *center*) and healthy fruit. **C** Vertical section of infected and uninfected fruit. *Asterisk*, hyperplasia of tissue; *arrows*, abortive seed

Fig. 8. Symptoms on Exobasidium leaf blight on *S. lucida* by *E. symploci-japonicae* var. *symploci-japonicae.* **A** Infection occurred on the main branch of NIAES 20520. **B** Infection occurred on the lateral branch of NIAES 20521. *Bars* 10 mm

On infected fruit of *S. lucida*, white powdery hymenia were formed entirely on the swollen part of the fruit (Fig. 7A). Half the fruit were apparently bigger than the healthy ones and pale green or whitish-green (Fig. 7B). Sections of the infected fruit show hypertrophy and hyperplasia. Hyphae spread intercellularly and were branched. Haustorium was not determined in these hand sections. All seed were sterile (Fig. 7C). Hymenium formation was not observed on leaf, twig, and branch as appeared in the symptoms caused by var. *symploci-japonicae* (Fig. 8A,B). An infected fruit was overmatured and colonized by the secondary invaders. These fruits were decayed.

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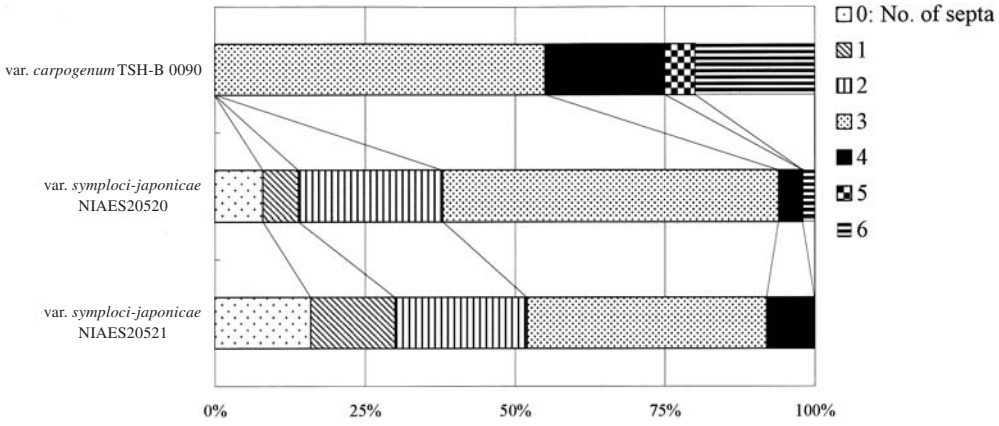

Fig. 9. Ratio of septal number in the basidiospores of *E. symploci-japonicae* var. *carpogenum* and var. *symploci-japonicae* NIAES 20520 and NIAES 20521

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