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Biological characteristics of *Cryptosporiopsis abietina* on Hinoki cypress and its antagonistic effect to other microorganisms

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In isolation tests on different parts of sound Hinoki cypress (*Chamaecyparis obtusa*), the coelomycete *Cryptosporiopsis abietina* was isolated, mostly from immature cone scales and inner bark areas of branches and main stems. In isolation tests using branches or stems of 3-yr-old seedlings to 35-yr-old adult cypresses from various localities, *C. abietina* was isolated in 28 to 100% of the cypresses tested. It was also confirmed that the fungus rarely infects seeds of the cypress, and can survive in dried seeds for several years, though it is not a seed-borne fungus. No symptoms appeared in inoculation tests with the fungus on 8-yr-old cypress. In inoculation tests in a greenhouse, reliable infection without symptoms was obtained on wounded cypress seedlings. These results reveal that *C. abietina* is a common endophytic fungus in Hinoki cypress. Confrontation and volatile antagonistic tests in Petri dishes indicated that some isolates of *C. abietina* produce antifungal substances in vitro. On these isolates, the reproduction of a mycophagous nematode *Bursaphelenchus xylophilus* was retarded. The results of inoculation tests suggest that the possibility of *C. abietina* as a primary causal agent of the resinous stem canker of Hinoki cypress is low. The role of *C. abietina* in the cypress is briefly discussed.

Key Words——antagonism; *Cryptosporiopsis abietina*; endophytic fungus; mycophagous nematode; *Pezicula livida*; resinous stem canker.

Cryptosporiopsis abietina Petrak (teleomorph=*Pezicula livida* (Berk. et Br.) Rehm) has been reported as the causal organism of resinous stem canker of Hinoki cypress, *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl. (Kobayashi et al., 1990). However, the development of typical symptoms of the disease has not been confirmed by inoculation experiments with the fungus.

Cryptosporiopsis abietina also has been reported to be pathogenic to hosts (Kowalski, 1982; Haug et al., 1988). However, the species has been isolated as an endophytic fungus mostly from sound conifers and rarely from broad-leaved trees in Europe and North America (Carroll et al., 1977; Sieber and Hugentobler, 1987; Espinosa-Garcia and Lagenheim, 1990; Kowalski and Kehr, 1992, 1996; Rollinger and Lagenheim, 1993). Antagonistic traits of *C. abietina* (Pratt, 1982) and *Cryptosporiopsis* sp. (Ohsawa and Katsuya, 1987) against butt rot fungi have been reported.

The distribution and life cycle of the fungus and its function in Hinoki cypress have not been clarified. To know the pathogenicity and biological characteristics of *C. abietina*, the distribution of the fungus in Hinoki cypress and its antagonistic effects on other pathogenic fungi from the cypress were studied in vitro. Moreover, reproduction of the mycophagous nematode *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, the causal agent of the pine wilt disease, on this fungus was compared with that on *Botrytis cinerea* Pers.: Fr., which is known as a medium for culturing the nematode. Based on the results of inoculation tests with the fungus, the role of the fungal species under field conditions was discussed.

Materials and Methods

Isolation tests on different parts of sound Hinoki cypress

To clarify the distribution of *C. abietina* in different parts of adult Hinoki cypress, isolation samples were collected from five 20-yr-old cypresses planted at the experimental forest of the Forestry and Forest Products Research Institute (FFPRI), in Kukizaki, Ibaraki Pref. in December 1992. Isolations were conducted on matured seeds, immature cone scales, current-year leaves, inner bark and xylem of a 3-yr-old branch at approximately 2 m height, and inner bark of the stem at 1.5 m height of individual trees. For surface-sterilization, the samples were dipped in 70% ethanol for 1 min, then in 10% sodium hypochlorite (commercial Antiformin, Wako Chemical) for 5 min. After rinsing in sterilized water and drying on a sterilized filter paper, the samples were then cut into segments of approximately 5×3 mm. Five segments were placed in a single plate containing PDA (Eiken

Chemical), and five replicates were made. Percent isolation frequencies of *C. abietina* among cypress trees tested and among total segments tested were recorded. For the isolation from seeds, whole seeds were used without dividing them into smaller segments, and the period of ethanol dipping was shortened to 10 s. Plates were incubated at 15°C in darkness.

Isolation tests on stems and branches of sound Hinoki cypress from various localities Isolation tests of *C. abietina* from 2-mo-old seedlings to 35-yr-old adult cypresses were conducted on samples from various localities, including Beppu, Oita Pref.; Kuzuo, Tochigi Pref.; Ogawa, Ibaraki Pref.; and the FFPRI, Ibaraki Pref.

For the isolation from the stems of adult trees, five healthy trees were chosen at a plantation in Tochigi Pref. Materials including inner bark were collected from two areas of stems at 1.5 m in height. For the isolation from the branches, a branchlet was collected from individual trees randomly chosen at each site, and parts of the 3-yr-old were chosen for isolation samples at the laboratory. The inner bark of individual samples was surface sterilized and used for the isolation as described above. For the isolation from 3-yr-old seedlings, main stems were used without removing their outer bark. The stems of 2- and 5-mo-old seedlings were used for the isolations without dividing them into smaller segments and without removing their outer bark.

Isolation tests on stocked seeds Twenty-one seed stocks of Hinoki cypress which were collected from various localities and preserved at 8°C at the FFPRI were used for this experiment. Seeds were surface sterilized in the same manner as the fresh seeds. One hundred seeds were processed for each seed stock.

Inoculation tests on 8-yr-old Hinoki cypress in a nursery *Cryptosporiopsis abietina* (C29) cultured for 2 mo in a sawdust medium containing rice bran at 20°C was used as inoculum. A 5-cm-long wound was made between the bark and xylem of the stems of 10 8-yr-old Hinoki cypresses on 2 Nov. 1992, and the inoculum was inserted into the wounds. The wounds were wrapped completely with vinyl tape. Ten additional cypresses served as controls, being inoculated with a fungus-free medium.

Inoculation tests on 1-yr-old seedlings in a greenhouse For the experiments, 1-yr-old, greenhouse-grown seedlings of Hinoki cypress were transplanted in March 1992 in clay pots (14 cm in diam), three per pot. These were kept in a greenhouse of the FFPRI throughout the experiment. Inoculations were done on 30 October 1992. Just before inoculation, the stem base of 30 seedlings was slightly wounded with sandpaper, and 30 other seedlings were inoculated without being wounded. Thirty additional seedlings served as controls. As inoculum, conidia of *C. abietina* (C31) produced in 2-mo-old cultures on PDA plates were used; after filtering off mycelial debris of cultures, conidia were diluted with distilled water and sprayed on the seedlings. After inoculation, the seedlings were placed in a moist chamber for 2 d at 20°C, then transferred to the greenhouse.

To confirm the colonization of the inoculated fungus, isolations were attempted from the stems of the seedlings 3.5 yr after inoculation. The stem of a seedling was divided into five equal parts. One 1-cm-long segment from the basal position of each part was used for isolations without removing outer bark.

Test of antagonism in paired cultures The antagonistic effect of 11 isolates of *C. abietina* (Table 1) against *Guignardia cryptomeriae* Sawada, the cause of Guignardia dieback of Hinoki cypress, was compared on PDA in 90-mm Petri plates. *Cryptosporiopsis abietina* was inoculated with 5-mm agar plugs at both ends of a line running through the plate center and allowed to grow at 20°C in darkness. Three days later, 5-mm mycelial disks of *G. cryptomeriae* (GC61) were placed in the center of the plates, which were then incubated under the same conditions. The width of two inhibition zones formed between the two fungal species in a plate and radial growth of both species were measured 9 d later. Six replicates were made for each isolate of *C. abietina*.

Volatile antagonistic test The effect of volatile substances produced by *C. abietina* on the growth of *G.*

Table 1. Isolates of *Cryptosporiopsis abietina* from *Chamaecyparis obtusa*.

Isolate no.	Parts from which *C.a.* was isolated	Condition of cypress[a)]	Mo and yr of isolation	Locality
C20	Seed	N	April 1992	Ibaraki
C21	Inner bark of stem	N	July 1992	Ibaraki
C22	Seed	N	April 1992	Ibaraki
C23	Cone scale	N	Dec. 1992	Ibaraki
C24	Cone scale	N	Dec. 1992	Ibaraki
C25	Cone scale	N	Dec. 1992	Ibaraki
C26	Seed	N	Dec. 1991	Ibaraki
C28	Inner bark of stem	R	June 1991	Tokyo
C29	Inner bark of stem	R	Sept 1992	Tochigi
C30	Inner bark of branch	N	July 1991	Ibaraki
C31	Inner bark of stem	R	June 1991	Ibaraki

a) Condition of cypress from which the fungus was isolated; N, no symptom; R, affected by resinous stem canker.

cryptomeriae and *Seiridium unicorne* (Cke. et Ell.) Sutton, the cause of Seiridium canker of Hinoki cypress, was investigated in vitro. Inoculum discs (5 mm in diam) of *C. abietina* were inoculated at the center of the PDA plates (90 mm in diam). Two isolates, C28 and C30, were used for this experiment. The plates were incubated at 20°C in the dark. Four days later, a second set of Petri plates was inoculated with 5-mm mycelial plugs of *G. cryptomeriae* or *S. unicorne* at the center of the PDA plates. Immediately after inoculation, the upper lids of the plates containing *G. cryptomeriae* and *C. abietina* (4-d-old culture) were removed and the tops of two culture plates were joined. The tops of plates containing *S. unicorne* were also joined with the tops of the plates of *C. abietina*. The joined plates were wrapped with parafilm and incubated at 20°C. Control plates were prepared in the same way without inoculating *C. abietina* on the medium. Colony diam of the inoculated fungi were measured every day for 11 d.

Reproduction of the pine wood nematode on different isolates of *C. abietina* To compare the reproduction rate of the pine wood nematode *Bursaphelenchus xylophilus*, a mycophagous nematode and the causal agent of pine wilt disease, 11 isolates of *C. abietina* (Table 1) were allowed to grow on PDA Petri plates (60 mm in diam) at 20°C in the dark. When colonies covered the entire surface of the plates, a droplet of sterilized distilled water containing 100 nematodes (S6-1) was placed on the plates. The number of nematodes was calculated after incubating the plates for 12 d at 28°C in the dark. Three replicates were made for each isolate of *C. abietina*. Control plates were prepared by inoculating the nematode onto the colonies of *B. cinerea* in the PDA plates.

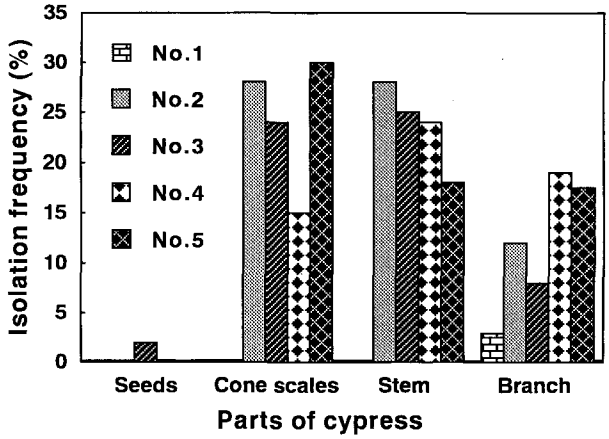

Fig. 1. Isolation frequency of *Cryptosporiopsis abietina* from different parts of five (nos. 1-5) 20-yr-old *Chamaecyparis obtusa*.

Results

Isolation tests on different parts of sound Hinoki cypress When five 20-yr-old, sound Hinoki cypresses planted in the experimental forest of the FFPRI were sampled, *C. abietina* was isolated from the inner bark of branches of all five trees with an isolation frequency of 3-19% among segments tested, and from immature cone scales (still showing green color) and the inner bark of stems of four of the trees at a frequency of 15-30% (Fig. 1). From matured seeds, *C. abietina* was isolated only from cypress no. 3 at a frequency of 3%. No fungus was isolated from the current-year leaves and xylem of 3-yr-old branches of any of the trees tested.

A sterile fungal species and *Pestalotiopsis* sp. on

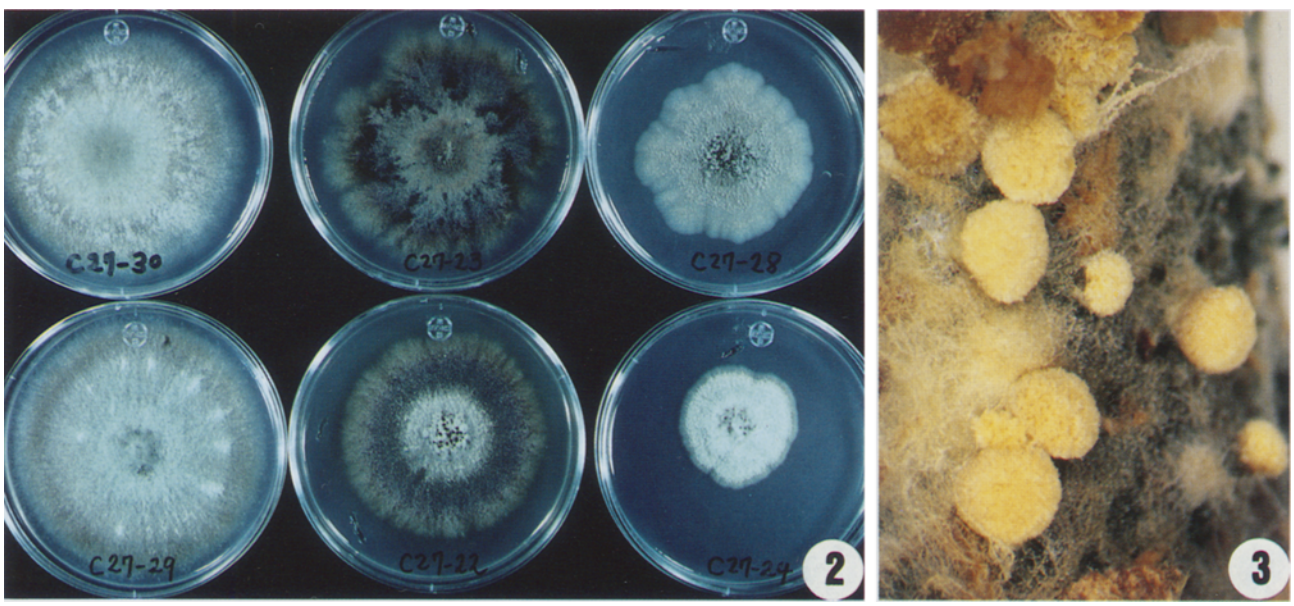

Fig. 2. Three colony types in color of *Cryptosporiopsis abietina*: green-yellow type (left), brown type (middle), and white type (right).
Fig. 3. Apothecia of *Cryptosporiopsis abietina* produced on sawdust medium.

Table 2. Isolation frequency of *Cryptosporiopsis abietina* from sound *Chamaecyparis obtusa*.

Locality	Age of cypress	Number of cypress tested	Parts of cypress	Isolation frequency among cypresses tested (%)	Isolation frequency among segments tested (%)
Beppu, Oita (plantation)	35-yr	10	Branch	70	22
Kukizaki, Ibaraki (expt. forest)	20-yr	10	Branch	80	8
Ogawa, Ibaraki (plantation)	10-yr	20	Branch	85	24
Kuzuo, Tochigi (plantation)	20-yr	5	Stem	100	19
Kukizaki, Ibaraki (nursery)	3-yr	18	Stem	28	8
Kukizaki, Ibaraki (greenhouse)[a)]	5-mo	50	Stem	0	0
Kukizaki, Ibaraki (greenhouse)[a)]	2-mo	60	Stem	0	0

a) *Cryptosporiopsis abietina* was isolated (4–17%) from the seeds when tested before seeding.

cone scales, an unidentified species which seems to be a discomycetous fungus on seeds, an unidentified hyphomycete, and a sterile species on the inner bark of stems and branches were more dominant.

The colonies of the isolated *C. abietina* were divided into three types by color: green-yellow, brown, and white (Fig. 2). No clear distinction was found in the colony types among the samples used for the isolation test. Neither were any distinctions found in the morphology of conidia among the three colony types. Apothecia of the *Pezicula* state were produced in cultures on a sawdust medium and PDA (Fig. 3).

Isolation tests on stems and branches of sound Hinoki cypress from various localities *Cryptosporiopsis abietina* was isolated from the branches of 10-, 20-, and 35-yr-old cypresses and stems of 20-yr-old cypresses with high frequency among the trees tested, though the isolation frequency among segments tested was lower (8–24%) (Table 2). From the main stems, *C. abietina* was detected in all five of the 20-yr-old trees and in 28% of 3-yr-old seedlings tested. When the stems of 2- and 5-mo-old seedlings were tested, the fungus was not isolated, though it had been isolated with 4–17% frequency from the seeds when tested before seeding.

The species composition of other fungi differed depending on the sample.

Isolation tests on stocked seeds Isolation tests on six seed stocks were conducted in 1990 and 1991 (Table 3). In these tests, *C. abietina* was isolated only from the seeds of stock no. 88-026 collected in Ibaraki Pref. in 1988 with an isolation frequency of 17% in 1990 and 3% in 1991. In the germination rates of seeds, no clear distinction was found between no. 88-026 and the other stocks.

In June 1993, further isolation tests were conducted on 21 seed stocks including 6 stocks used in the previous tests. Eight of these stocks had been collected in 1978, 3 in 1988, and 10 in 1991. In this experiment, the fungus was not isolated from any of the seed stocks.

From stocked seeds, the isolation frequency of such other fungi as *Phoma* sp., *Colletotrichum* sp. or *Pestalotiopsis* sp. was 3–10%.

Inoculation tests on 8-yr-old Hinoki cypress in a nursery No differences were found in symptom development between the stems of inoculated trees and controls within 3 yr after inoculation, and the wounds healed over by callus formation.

Inoculation test on 1-yr-old seedlings in a greenhouse No symptoms appeared on the inoculated seedlings and controls within 3.5 yr after inoculation.

In isolation tests on inoculated seedlings 3.5 yr after inoculation, *C. abietina* was reisolated from all 30 seedlings whose stem bases had been slightly wounded with sandpaper just before inoculation (Fig. 4). The average isolation frequency among segments was 56%. The fungus was detected in 20% of the seedlings inoculated without wounding with an isolation frequency of 20% among segments tested. From control seedlings, *C. abietina* was not isolated. From controls and the seedlings inoculated without wounding, *Papularia* sp. was isolated with 63% and 83% frequency among segments, respectively.

Table 3. Results of isolation tests of *Cryptosporiopsis abietina* from stocked seeds of *Chamaecyparis obtusa*.

Stock no. of seeds	Year of collection	Prefecture where seeds were collected	Isolation frequency of *C. abietina* (%)		Germination rate of seeds (%)	
			in 1990	in 1991	in Petri dish	in pot
78-001	1978	Kochi	0	0	6	1
78-013	1978	Nagano	0	0	46	26
78-018	1978	Nagano	0	0	18	8
88-026	1988	Ibaraki	17	3	22	13
88-030	1988	Ibaraki	0	0	52	23
88-051	1988	Kyoto	0	0	2	0.5

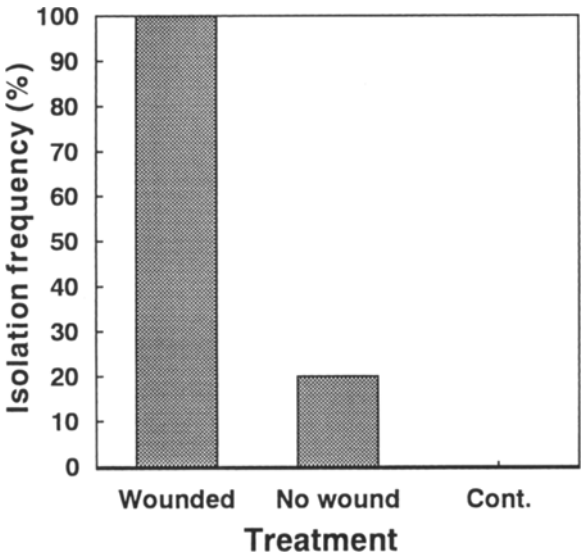

Fig. 4. Isolation frequency of *Cryptosporiopsis abietina* among 30 *Chamaecyparis obtusa* seedlings 3.5 yr after inoculation.

Test of antagonism in paired cultures The width of inhibition zones between *C. abietina* and *G. cryptomeriae* varied according to the isolate of *C. abietina* (Figs. 5, 6). The inhibition zones were prominent in the paired culture using *C. abietina* isolates C28, 31, 25, and 24, which were of either brown or white colony type. The green-yellow type (C20, 21, 29, and 30) tended to show little or no antagonistic effect against *G. cryptomeriae*. Inhibition zones were not formed between *G. cryptomeriae* and *C. abietina* isolates C21, 29, and 30. A strong negative correlation (correlation coefficient=0.839) was found between the colony diameter of *G. cryptomeriae* and the width of inhibition zones.

Volatile antagonistic test In the volatile antagonistic test, the presence of a colony of *C. abietina* significantly inhibited the radial growth of *G. cryptomeriae* (Fig. 7). The degree of inhibition was higher in the C28 isolate (brown type) than in the C30 (green-yellow type). The antagonistic effect against *S. unicorne* was less significant, though the colonies of *S. unicorne* apparently became thinner and paler in the presence of *C. abietina* (Fig. 8).

Reproduction of pine wood nematode on different isolates of *C. abietina* When the pine wood nematode was cultured on different isolates of *C. abietina*, the reproduction of the nematode on isolates nos. C20, 21, 26, 29, and 30 was not greatly different from that of the control (on *B. cinerea*) (Fig. 9). However, on isolates C22, 23, 24, 25, 28, and 31, the reproduction of the nematode was significantly suppressed compared to the control.

Discussion

In Japan, fruit-bodies of *P. livida*, the teleomorph of *C. abietina*, have been recorded on dead branches of larches (*Larix* spp.) and pines (*Pinus* spp.) (Saho and Takahashi, 1973; Kobayashi et al., 1990) and on the lesions of resinous stem cankers of Hinoki cypress (Kaneko et al., 1985; Kobayashi et al., 1990). The *Cryptosporiopsis* state has been isolated from lesions with resin flow, which were formed by various causes on Hinoki cypress, Sawara cypress (*Ch. pisifera* (Sieb. et Zucc.) Endl.), Japanese cedar (*Cryptomeria japonica* (L.) D. Don), and Japanese larch (*L. kaempferi* (Lamb.) Carriere) (Kobayashi et al., 1990). Shoji (1990) isolated this fungus from sound stems of eight tree species of Cupressaceae collected from the Kanto district in central Japan. In Europe and North America, *C. abietina* is a common endophytic fungus found mainly on conifers, but also less frequently on some broad-leaved trees.

The isolation tests of stems and branches of sound Hinoki cypresses collected at various localities revealed that *C. abietina* is an endophytic common colonizer in inner bark areas and cone scales of Hinoki cypress. The colonization rate of *C. abietina* on sound cypress was apparently not different from that on cankered Hinoki cypress, when the isolation frequency in the present study was compared with that on cankered cypresses by us (unpublished data) and by Kobayashi et al. (1990).

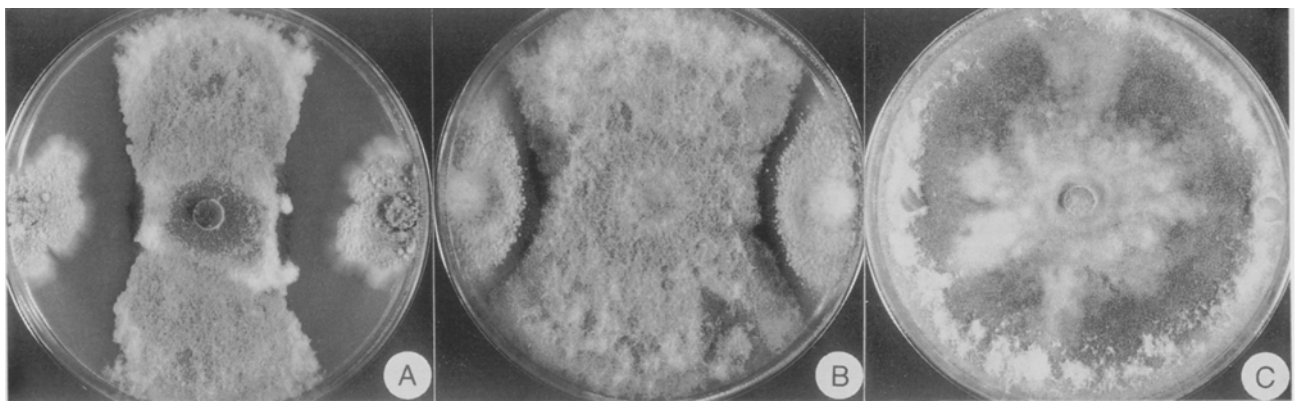

Fig. 5. Inhibition of *Guignardia cryptomeriae* (center of plate) by *Cryptosporiopsis abietina* (both ends of plate) in paired culture. A. Wide inhibition zones between *C. abietina* (C28) and *G. cryptomeriae*. B. Narrow inhibition zones between *C. abietina* (C29) and *G. cryptomeriae*. C. Control (developed colony of *G. cryptomeriae*).

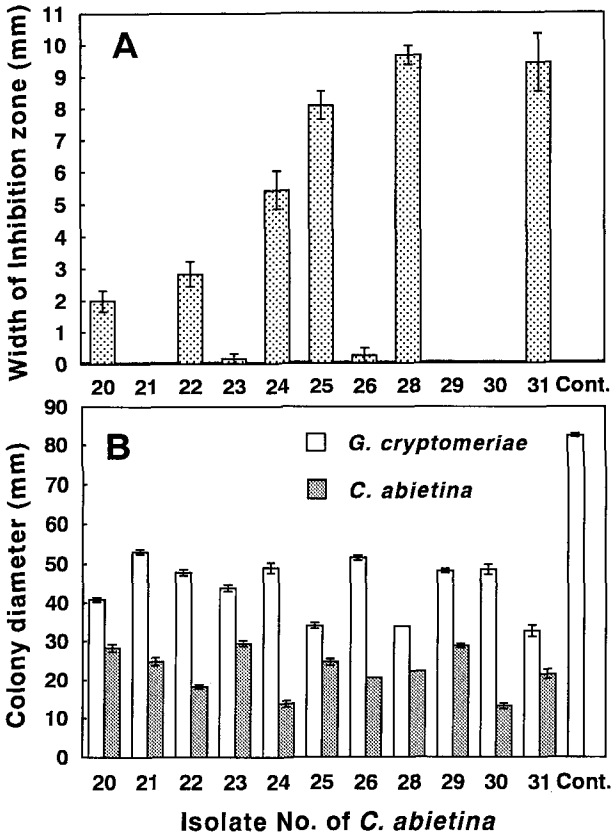

Fig. 6. Antagonism in paired culture. A. Width of inhibition zone between different isolates of *Cryptosporiopsis abietina* and *Guignardia cryptomeriae*. B. Growth of *G. cryptomeriae* colony in paired culture with different isolates of *C. abietina*. Bars=SE.

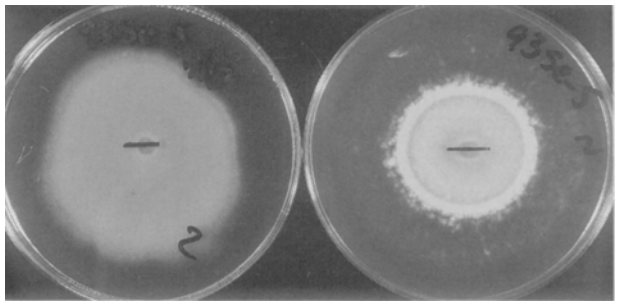

Fig. 8. Growth of *Seiridium unicorne* showing volatile antagonistic effect by *Cryptosporiopsis abietina* (C30) (right) and control (colony of *S. unicorne* grown without *C. abietina*) (left).

The fungus may also be common in other conifers in Japan, because in our preliminary experiments it was isolated from the branches of sound Japanese red and black pines and Japanese cedar.

In our study, *C. abietina* was rarely detected inside the seeds of living cypresses in a forest and of a certain seed stock. However, the fungus is probably not a seed-borne fungus, because it was not isolated from the seedlings grown from the seed stock, from which the fungus had been isolated. It is also believed that *C. abietina* can survive inside seeds for only a few years, because the isolation frequency of the fungus gradually decreased in these tests. No clear tendency was found in the germination rate of seeds between the stock where the fungus had been isolated and the other stocks. This suggests that *C. abietina* existing in cypress seeds probably does not affect the viability of seeds, though more data are required before a firm conclusion can be reached.

In the inoculation tests on 1-yr-old seedlings in a

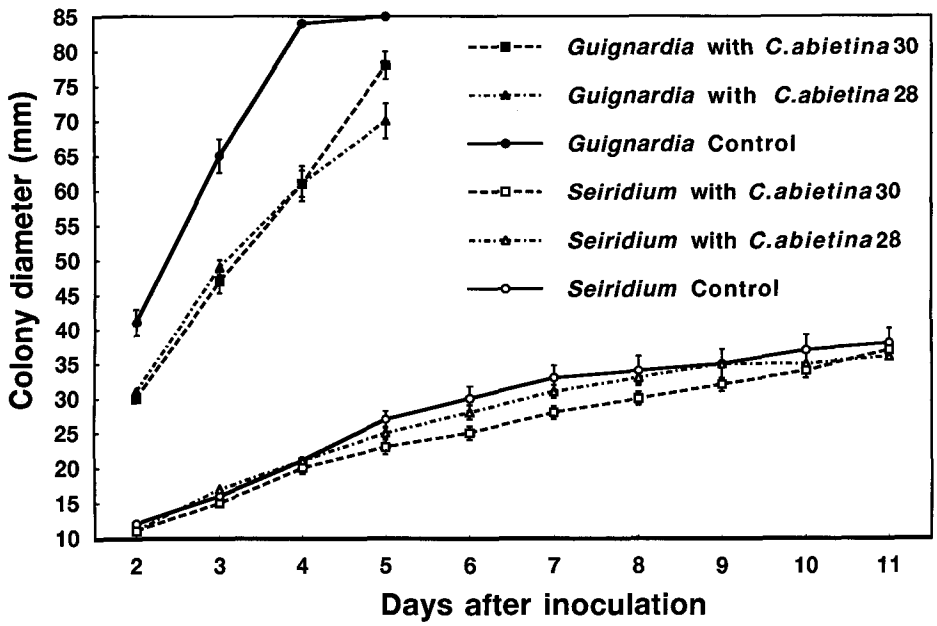

Fig. 7. Growth of colonies of *Guignardia cryptomeriae* and *Seiridium unicorne* in volatile antagonistic test. Bars=SE.

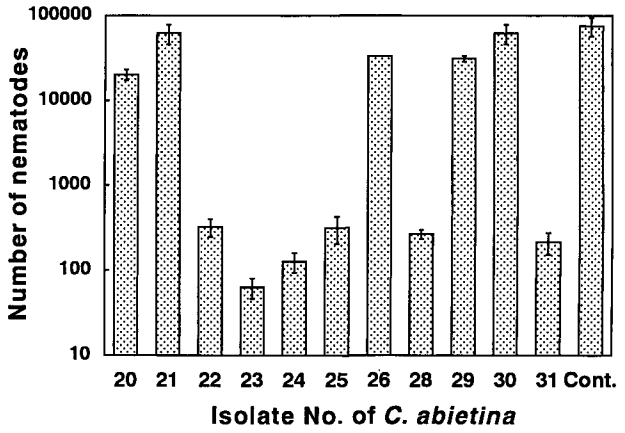

Fig. 9. Reproduction of *Bursaphelenchus xylophilus* on different isolates of *Cryptosporiopsis abietina*. Bars=SE.

greenhouse, *C. abietina* was reisolated from almost all stem-heights of all the seedlings which had been inoculated just after wounding. On the other hand, the fungus was rarely isolated from seedlings which had been inoculated without wounding. All uninoculated seedlings kept in a greenhouse remained uninfected by the fungus. This evidence reveals that *C. abietina* enters into the cypress through wounds, and its mycelia can probably grow in the inner bark area. Under natural conditions, however, the infection may also occur through juvenile shoots or cones. Further experiments are needed to confirm this possibility. Concerning spore states of the fungus, the anamorph produced on host plants has not yet been found in Japan, though the *Pezicula* state is rather common on dead branches and cankered lesions. This suggests an important role of ascospores in the dispersal of the fungus under natural conditions.

Some endophytic fungi from woody plants are suggested to have antagonistic effects on pathogenic fungi (Petrini, 1991) or harmful insects (Sieber and Hugentobler, 1987; Johnson and Whitney, 1989). Confrontation and volatile tests in Petri dishes in our study indicated that *C. abietina* produces antifungal substances in vitro. This may include both water-soluble and volatile substances, and the ability to produce antifungal substances may differ depending on the fungal isolate. A comparison of colony types showed that the green-yellow type seemed to have little or no antifungal effect.

In terms of reproduction of the mycophagous pine wood nematode, *C. abietina* could be divided into two groups. One group comprised mostly of the green-yellow colony type reproduced to the same degree as on *B. cinerea*, the control. In the other group, however, the reproduction of the nematode was retarded significantly. A comparison between the reproduction of the nematode on different isolates and the antifungal effect of these isolates showed a negative correlation (95% level; correlation coefficient $=-0.672$). This indicates that similar substance(s) may be involved in both the suppression of the nematode reproduction and the antifungal effect.

Antifungal substances have been isolated from *Cryptosporiopsis* species containing *C. abietina* (Stilwell et al., 1969; Fisher et al., 1984; Noble et al., 1991; Schultz et al., 1995). Our group (Yada et al., 1994) isolated a new sterol from mycelia of *C. abietina* and determined its structure. It has abscisic activity against Hinoki cypress leaves. Also, some phenol compounds have been isolated from culture filtrates and mycelia of *C. abietina* (H. Sato, personal communication). The effects of these substances on other fungi, insects and host trees must be clarified to understand the significance of *C. abietina* in sound cypress. It must also be confirmed whether the fungus provides sufficient quantities of such antibiotic compounds in living trees to show antagonistic effects against pathogenic fungi or insects.

Our study indicated clearly that we are able to prepare *C. abietina*-infected and -uninfected seedlings, a simple but important finding. By employing this technique, the effects of *C. abietina* as an endophytic fungus against more aggressive pathogenic fungi or harmful insects should be studied in vivo. In addition, the exact tissue where *C. abietina* exists and how it can live in trees needs to be clarified.

Cryptosporiopsis abietina has been reported as the causal fungus of the resinous stem canker of Hinoki cypress (Kobayashi et al., 1990). Slight canker formation following inoculation with *C. abietina* has also been reported (Hayashi and Kobayashi, 1985; Yokozawa et al., 1986; Sakuyama et al., 1987). However, further development of the symptoms into typical resinous stem canker has not been reported by these authors. In the present study, no symptom appeared in the inoculation tests on 8-yr-old and 1-yr-old cypresses. No clear symptoms appeared also in our inoculation tests on adult Hinoki cypress (unpublished data). These findings suggest that *C. abietina* has no clear pathogenicity to the Hinoki cypress. Inoculation tests with a European *Pezicula* species, *P. cinnamomea* (DC.) Sacc., showed it to be a weak parasite of red oak (*Quercus rubra*) causing cambium necrosis during the dormant season (Kehr, 1991). In this case, drought or some other factor was considered to act a predisposing factor for the onset of the disease. The same author (Kehr, 1992) indicated that *P. cinnamomea* is a common endophyte of red oak, and development of Pezicula canker is dependent on the reduction of host vigor. *Cryptosporiopsis abietina* is also considered to be a pathogen of a canker of weakened larch (Kowalski, 1982). *Cryptosporiopsis abietina* has severely infected the vascular tissue of *Picea abies* seedlings grown sterilely in Petri dishes and caused the quick decline of spruce seedlings without mycorrhizae (Haug et al., 1988). This apparent pathogenicity in tree tissues contradicts the findings of many other reports, probably because the seedlings were grown sterilely in vitro.

From the results of our and other inoculation tests, the possibility that *C. abietina* is a primary causal agent of the resinous stem canker of Hinoki cypress seems low, though the abscisterol from the fungus (Yada et al., 1994) may have some stimulatory effects on the cypress trees. Suto (1992) reported *Cistella japonica* Suto as a new candidate pathogen of the resinous stem canker of Hinoki cypress. As stated by Carroll (1988), or as in the

case of the Pezicula canker of Red oak in Europe (Kehr, 1991), many stem and leaf pathogens may have latent endophytic stages in their life cycles. In the process of symptom development of the resinous stem canker, the death of cambium zones results in the formation of cankers, usually several years after the first resin-exudate appears in affected lesions. In the case of the resinous stem canker of Hinoki cypress, the entire affected tree is probably not in a condition of reduced vigor, because the diameter growth of trees is usually not suppressed. However, the partial death of cambial zones will create stress in limited areas. In this case, we need to clarify whether *C. abietina* acts as a weak pathogen after the first resin-exudate appears or completes its life cycle without showing any pathogenicity in host plants. In this regard, slight differences have been observed in the recovery of artificial wounds between *C. abietina*-inoculated and control trees in inoculation tests during the dormant season of cypresses (Hayashi et al., 1985; Yokozawa et al., 1986; Sakuyama et al., 1987).

Kowalski and Kehr (1996) suggested the significance of endophytic fungi in the process of natural pruning of tree branches. An endophytic fungus may help the tree to shed branches while protecting the tissues of the main stem against more aggressive fungi. Kaneko (1992) found an endophytic *Asteroma* species in immature acorns of Japanese beech. The fungus caused the premature falling of the acorns invaded by insects. In this case the fungus may help the tree by preventing the migration of nutriment into useless acorns, though the acorn itself suffers disease. These are examples of "beneficial disease for host" caused by an endophytic fungus. *C. abietina* produces a sterol with abscisic activity. This invites speculation that *C. abietina* in sound cypresses may have an important role in natural pruning of branches. This role also needs to be clarified.

Regarding the taxonomy of *C. abietina* and related species, no differences were found in the morphological characteristics of *P. livida* among Japanese collections and North American specimens at the BPI (U.S.A.) and DAOM (Canada) (Kaneko et al., 1985). No morphological differences exist among isolates with different colony types. The same finding was noted by Kobayashi et al. (1990). Isozyme and DNA analysis of various isolates including ours, however, revealed considerable distinctions (Jensen and Petrini, 1994). The Japanese isolates we provided were shown to be a uniform group. In the future, the taxonomical position of *C. abietina* and its teleomorph *P. livida* from Japan should be reexamined by means including molecular analysis.

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