Ecology and biodiversity of *Halophytophthora* species

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The ecology and diversity of *Halophytophthora* species has been investigated at the Shiira River, Iriomote Island, Japan. The frequency of occurrence of *Halophytophthora* species in the subtropical mangrove along the river was examined during different seasons by means of the leaf-bag method. Senescent yellow leaves of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* were placed in mesh bags and submerged for two days at four stations along the river, from the upper stretches to the river mouth. *Halophytophthora* species that appeared on disks cut out from retrieved leaves and incubated on seawater cornmeal agar plates, were recorded and isolated. Naturally fallen leaves of various mangrove trees were also collected to examine the occurrence of *Halophytophthora* species. These experiments showed that (i) *H. vesicula* occurred most frequently at all the stations during both cold and warm seasons, (ii) *H. spinosa* var. *lobata* appeared frequently at the lower river and river mouth sites, especially at during the warm season, and abundantly on *B. gymnorrhiza* leaves, but scarcely from *R. stylosa* leaves, (iii) *H. kandeliae* appeared only at the upper river site and on *R. stylosa* leaves, (iv) mycoparasitic *Halophytophthora* species (*H. mycoparasitica* and *H. epistomium*) appeared at a low frequency only in the cool season, in association with their hyphomycete hosts, (v) *H. avicennae* was found only from fallen leaves of *Avicennia marina* inhabiting the river mouth, and (vi) *Halophytophthora* sp. (a new species) was found repeatedly, but only on submerged leaves of *Sonneratia alba*, which inhabited the river mouth. Observations on the growth and reproduction of *Halophytophthora* species revealed that they are well adaptated to the environmental conditions of their respective habitats. The levels and fluctuations of water salinity and temperature, and substrate types were found to be important factors influencing the local, seasonal and geographical distribution of *Halophytophthora* species.

Key words: biodiversity, ecology, *Halophytophthora*, mangroves, oomycetes.

Introduction

Halophytophthora species are zoosporic oomycetes that predominantly inhabit brackish water mangroves. The first species was described by Anastasiou and Churchland (1969) as *Phytophthora vesicula* from submerged leaves in Vancouver, Canada. Fell and Master (1975) discovered that submerged mangrove leaves provided an excellent substrate for these oomycetes and described four new species and two varieties from various parts of the world. Since then, several species have been described from mangroves in Australia, Japan, Taiwan and the United States (Pegg and Alcorn, 1982; Gerettson-Cornell and Simpson, 1984; Ho *et al*., 1991, 1992; Nakagiri *et al*., 1994). To date, 13 species and 2 varieties have been described in the genus. *Halophytophthora* was established by Ho and Jong (1990) transferring all *Phytophthora* species inhabiting saline water to the new genus.

The ecology of *Halophytophthora* species has been studied from various viewpoints. Fungal succession studies showed that *Halophytophthora* species are the first colonizers of fallen mangrove leaves (Newell *et al*., 1987; Nakagiri *et al*., 1989). After the leaves fall, zoospores of *Halophytophthora* species soon invade the submerged leaves. The cellulolytic activity of a *H. vesicula* (Anastasiou and Churchland) Ho and Jong has been demonstrated by laboratory experiments (Raghkumar *et al*., 1994). *Halophytophthora* species play an important role as decomposers, as well as producers. When fallen leaves are submerged in water, nutrients from the leaves rapidly leach out. *Halophytophthora* species rapidly colonize the leaves and their mycelia spread quickly within the leaf tissues, so that the nutrients are fixed in the leaves as mycelial proteins. By this process and activities of other fungi and microorganisms, the fallen leaves are transformed into a food source that is utilized by animals such as snails, crabs and zooplankton (Fell and Master, 1980). *Halophytophthora* species and other fungi are therefore decomposers and also secondary producers in the mangrove ecosystem. Studies on the growth and reproduction of *Halophytophthora* species in culture indicate that they are generally well adapted to the temperatures and salinities of mangrove brackish water (Nakagiri, 1993). Floristic data on species of *Halophytophthora* has been accumulated from various parts of the world (Newell, 1992; Singh and Steinke, 1992; Nakagiri, 1993; Tan and Pek, 1997), while seasonal distribution of species has been observed in subtropical mangroves (Nakagiri *et al*., 1996). Competition among species of *Halophytophthora* and with other fungi or bacteria was investigated by Newell and Fell (1997). They found that *H. vesicula* was the strongest competitor. Rapid colonisation by zoospores and mycelial occupation of leaves was also investigated by Newell *et al*. (1987) and Newell and Fell (1995). Chemotactic behavior of the zoospores was investigated by Leano *et al*. (1998). Thus, the ecology and diversity of *Halophytophthora* species is now fairly well known.

The following questions however, have yet to be answered: (i) What environmental factors affect the distribution of the *Halophytophthora* species? and (ii) How do *Halophytophthora* species adapt to their habitats? To answer these questions, a field and laboratory study was initiated. In the field, the leaf-bag method was employed to establish the occurrence of the species at different

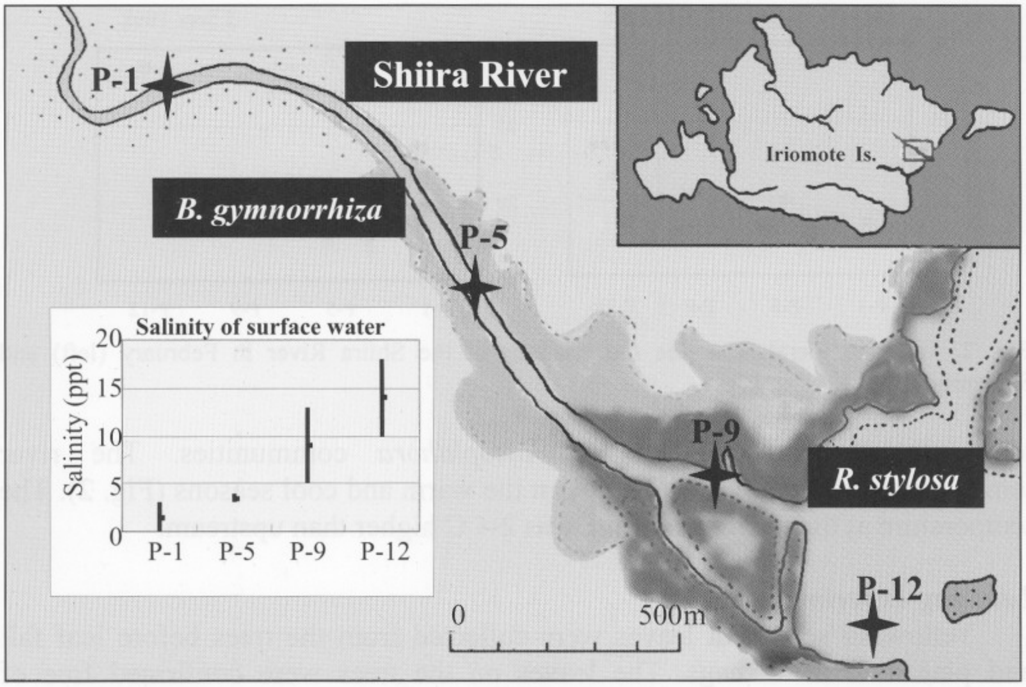


Fig. 1. Schematic illustration of the Shiira River in Iriomote Island. Four stations (Point-1, 5, 9 and 12) were set for the leaf bag experiments. The graph shows salinity level of the river water at the four stations (measured at the February experiment).

sites along a river and at different seasons. In the laboratory, growth and reproductive behaviour of each species was examined in order to establish the relationship between species occurrence and sites.

Materials and methods

Sites

Field studies were carried out along the Shiira River, Iriomote Island (24ºN, 124ºE), Japan in February and September in 1998. A schematic illustration of the Shiira River in Fig. 1 shows the zonation of the mangrove vegetation. *Rhizophora stylosa* occurs along the river mouth while *Bruguiera gymnorrhiza* occurs midstream to upstream. Four stations were selected. Point 1 was the upstream site, Point 5 the midstream site, Point 9 the downstream site and Point 12 the mouth of the estuary (Fig. 1). The salinity of the surface water of the river is also shown in Figure 1. The upstream site was almost freshwater with a very low salinity, while the salinity was higher downstream.

The surface water temperature of the estuary is lowest in January and February at 20 C and highest in July and August at 28-29 C. The field study was therefore carried out in February and early September to investigate the

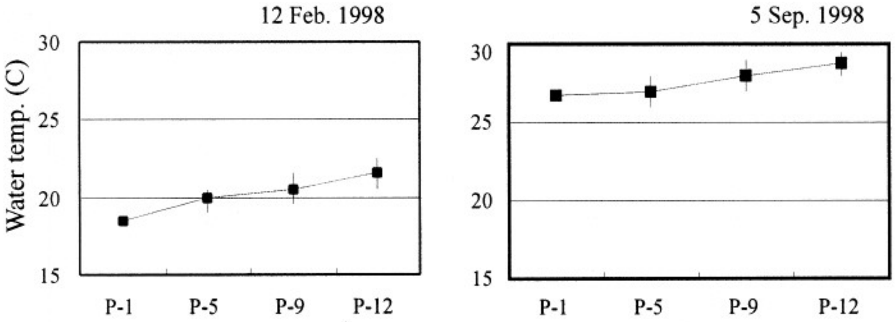


Fig. 2. Water temperature at the four stations of the Shiira River in February (left) and September (right).

temperature effect on the *Halophytophthora* communities. The river temperature differed by 6-9 C between the warm and cool seasons (Fig. 2). The temperature at the downstream site was 2-4 C higher than upstream.

Leaf bag experiments

Yellowing senescent leaves were collected from the trees before leaf fall and placed in mesh bags. The leaves on the trees were confirmed free of *Halophytophthora* species. Twenty leaves each of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* were placed inside separate bags and attached to the mesh with staples. The leaf bags were submerged in the river at the four stations. After 2 days of submersion, the leaf bags were retrieved. *Halophytophthora* species were detected and isolated by cutting disks from the submerged leaves with a cork borer. The leaf disks were washed in sterilised seawater, and incubated on cornmeal seawater (20 ppt salinity) agar (CMSWA). Thirty-two disks from each sample were examined. *Halophytophthora* species appearing from the disks were checked under the microscope and some of them were isolated for identification. Eumycota also appeared from the disks, but were not considered in this study. The frequency of occurrence of each *Halophytophthora* species was calculated as a percentage of the occurrence on the 32 disks.

Cultural studies

The hyphal growth and zoosporangium formation of each *Halophytophthora* species under different temperature and salinity regimes were examined in the manner reported previously (Nakagiri, 1993).

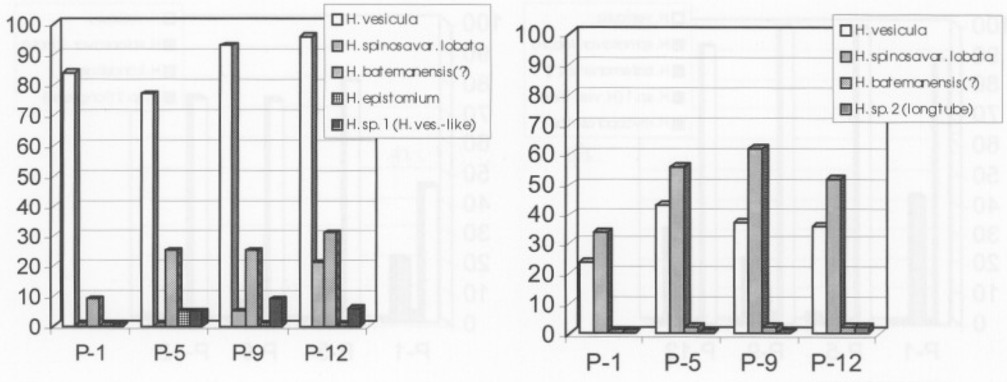


Fig. 3. Frequency (%) of occurrence of *Halophytophthora* species on *Bruguiera gymnorrhiza* leaves in February and September.

Results and discussion

Occurrence of species of Halophytophthora

The frequency of occurrence of *Halophytophthora* species on submerged leaves of *Bruguiera gymnorrhiza* at the four stations is shown in Figure 3. *Halophytophthora vesicula* was dominant at all the stations, especially in February. In September, however, *H. spinosa* var. *lobata* (Fell and Master) Ho and Jong was more prevalent over *H. vesicula*. *Halophytophthora spinosa* var. *lobata* occurred more frequently downstream and in the estuary, than at the upstream sites of the river. This was clearly seen in the February samples, as this species appeared only from Point-9 and Point-12 (Fig. 3).

The results from *Rhizophora stylosa* leaves (Fig. 4) showed that *Halophytophthora vesicula* occurred as the predominant species at all points of the river and at both cool and warm seasons. In contrast, *H. spinosa* var. *lobata* was scarce, even during the warm season. This marked difference from the data obtained from *Bruguiera gymnorrhiza* leaves may indicate that *Halophytophthora spinosa* var. *lobata* avoided *Rhizophora stylosa* leaves as a substrate. In comparing the data of the September experiments (see Figs. 3, 4), it is also clearly seen that *Halophytophthora vesicula* occurred more frequently on *Rhizophora stylosa* leaves than on *Bruguiera gymnorrhiza*. This may result from the lower competitive pressure among the *Halophytophthora* species on *Rhizophora stylosa* leaves, because of the absence of *Halophytophthora spinosa* var. *lobata*, thus enabling *H. vesicula* to occupy the *Rhizophora stylosa* leaves. The lower pressure of species competition might have also allowed *Halophytophthora kandeliae* Ho *et al.* to occur on *Rhizophora stylosa* leaves. This species appeared mainly upstream.

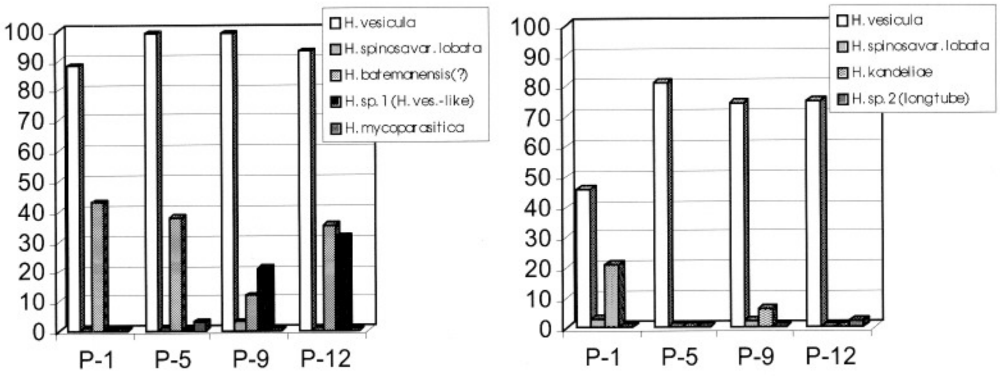


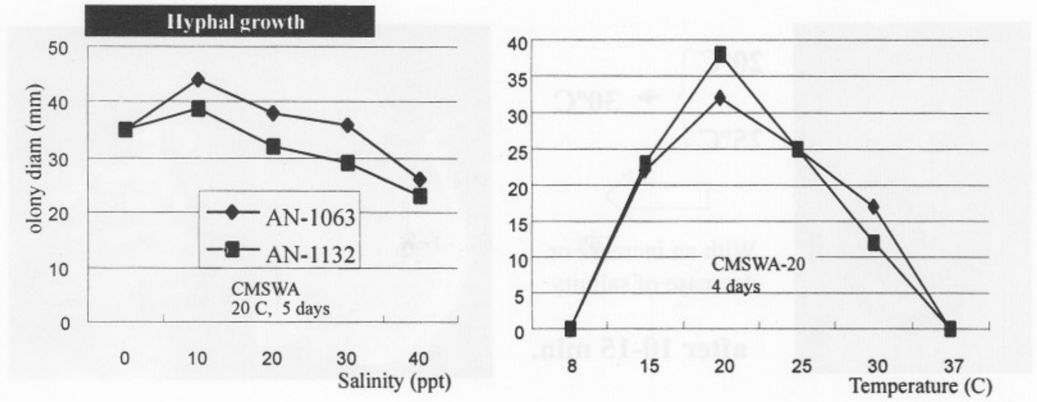
Fig. 4. Frequency (%) of occurrence of *Halophytophthora* species on *Rhizophora stylosa* leaves in February and September.

In the February but not September experiment, mycoparasitic species of *Halophytophthora* appeared at a low frequency. *Halophytophthora epistomium* (Fell and Master) Ho and Jong appeared on *Bruguiera gymnorrhiza* leaves, at midstream site in association with *Fusarium* or other hyphomycetes (Fig. 3). *Halophytophthora mycoparasitica* (Fell and Master) Ho and Jong appeared on *Rhizophora stylosa* leaves at the midstream (Fig. 4) and was found to be parasitic on *Pestalotiopsis* hyphae. This phenomenon may be attributed to the lower activity of host fungi and other saprobic *Halophytophthora* species during the cool season, thus allowing such slow growing mycoparasitic species to appear.

Growth and reproductive properties of **Halophytophthora** *species*

The growth and asexual reproduction of *Halophytophthora vesicula* under different salinity and temperature regimes are shown in Figure 5. The optimum salinity condition for mycelial growth was 10 ppt salinity, although it grows well at the range of 0 to 30 ppt. This species produced zoosporangia abundantly when the mycelia were submerged in water at all salinities examined, although the optimum was around 10-20 ppt. The mycelium grew well from 15 to 30 C, with the optimum at 20 C, and zoosporangia were formed at the range from 8 to 30 C. These results indicate that *H. vesicula* is adapted to a broad range of salinities and temperatures. These characteristics explain why *H. vesicula* was prevalent at all sites along the river, during both cool and warm seasons.

Halophytophthora spinosa var. *lobata* showed better growth at higher salinities of 20 to 40 ppt (Fig. 6). Zoosporangium formation was also better at higher salinities from 30 to 40 ppt. This species also grew well at higher temperatures. It could grow even at 37 C, a unique characteristic among the *Halophytophthora* species. This species produced zoosporangia well at 25-30



Zoosporangium formation

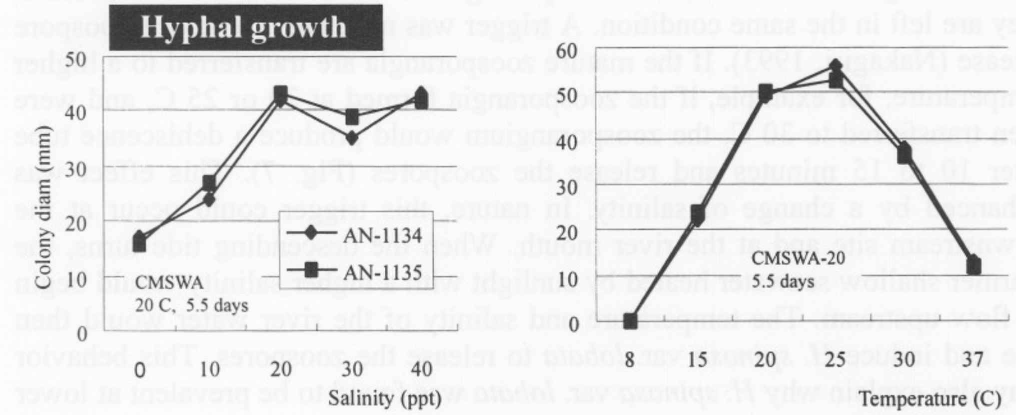
25 C, 1 day submerg.

	0	10	20	30	40 ppt
AN-1063	+	++++	++++	+++	++
AN-1132	+	++++	++++	+++	+++

20 ppt, 1 day submerg.

	8	15	20	25	30	37 C
AN-1063	+	++	+++	+++	+++	-
AN-1132	-	++	+++	++++	+++	-

Fig. 5. Hyphal growth (the upper graphs) and zoosporangium formation (the lower tables) of *H. vesicula* at different salinity (left) and temperatures (right).



Zoosporangium formation

25 C, 1 day submerg.

	0	10	20	30	40 ppt
AN-1134	-	+-	+	++	++
AN-1135	+-	+-	+	++	++

20 ppt, 1 day submerg.

	8	15	20	25	30	37 C
AN-1034	-	+	++	+++	+++	+
AN-1135	-	+	++	+++	+++	+

Fig. 6. Hyphal growth (the upper graphs) and zoosporangium formation (the lower tables) of *H. spinosa* var. *lobata* at different salinity (left) and temperatures (right).

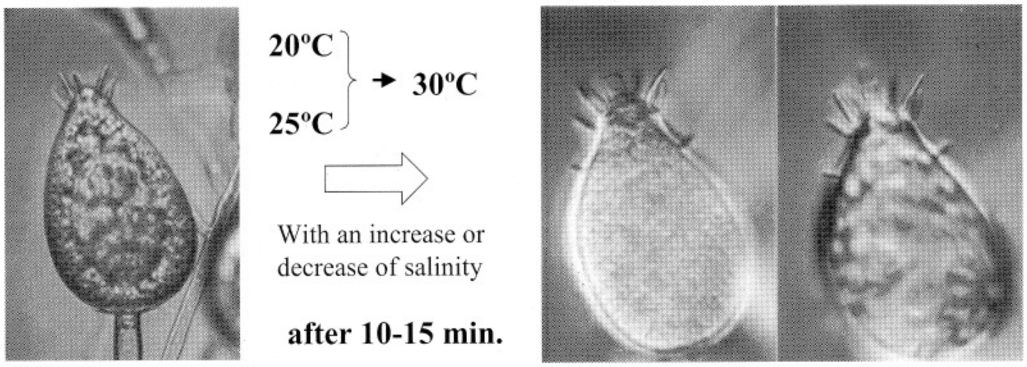


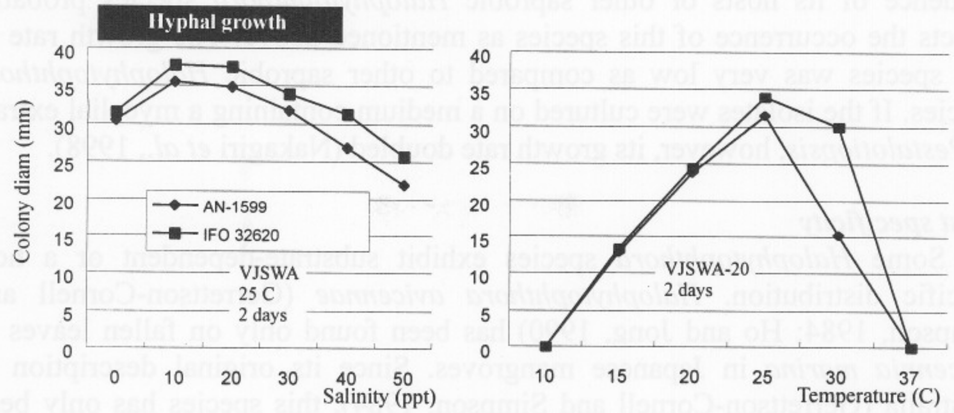
Fig. 7. Zoospore release of *H. spinosa* var. *lobata* triggered by a sudden increase of water temperature. Concurrent change of salinity enhances the temperature effect.

C, and in small numbers at 37 C. Thus, it appears to prefer higher salinities and temperatures. This may explain why this species mainly appeared during the warm season at the downstream sites, with its higher water salinity.

Halophytophthora spinosa var. *lobata* has a unique characteristic in zoospore release. This species produces many zoosporangia when the mycelia are submerged in seawater, but the sporangia do not release the zoospores if they are left in the same condition. A trigger was necessary to induce zoospore release (Nakagiri, 1993). If the mature zoosporangia are transferred to a higher temperature, for example, if the zoosporangia formed at 20 or 25 C, and were then transferred to 30 C, the zoosporangium would produce a dehiscence tube after 10 to 15 minutes and release the zoospores (Fig. 7). This effect was enhanced by a change of salinity. In nature, this trigger could occur at the downstream site and at the river mouth. When the descending tide turns, the warmer shallow seawater heated by sunlight with a higher salinity would begin to flow upstream. The temperature and salinity of the river water would then rise and induce *H. spinosa* var. *lobata* to release the zoospores. This behavior may also explain why *H. spinosa* var. *lobata* was found to be prevalent at lower stream sites and in the estuary.

Halophytophthora kandeliae has better growth and reproduction at lower salinities (around 10 ppt) and higher temperatures (25-30 C) (Fig. 8). The occurrence of this species upstream and during the warm season can be explained by this preference.

A mycoparasitic species, *H. mycoparasitica* grew and reproduced better at higher salinities (30-40 ppt) (Fig. 9). This species preferred relatively lower temperatures, because it showed better growth and reproduction at 20-25 C, while zoosporangium formation was impeded at 30 C (Fig. 9). The occurrence of this species only during the cool season may result from this preference. The



Zoosporangium formation

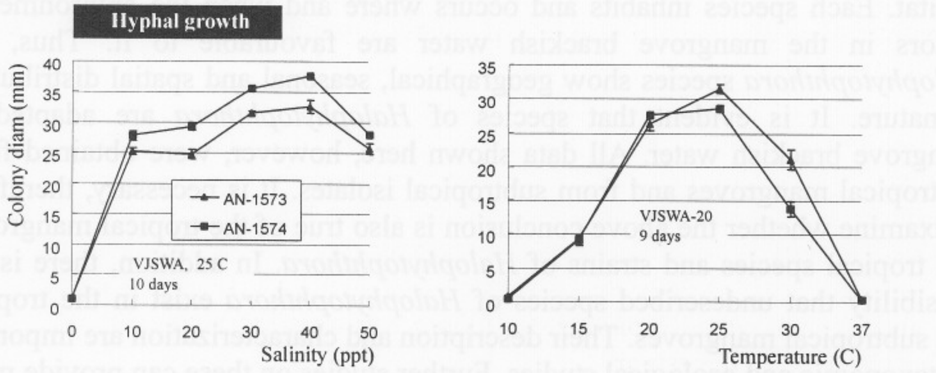
25 C, 1 day submerg.

	0	10	20	30	40 ppt
AN-1599	+-	++	+	-	-
IFO-32620	+	++	+-	+-	-

10 ppt, 1 day submerg.

	8	15	20	25	30	37 C
AN-1599	-	-	+-	++	+++	-
IFO-32620	-	-	-	++	+++	-

Fig. 8. Hyphal growth (the upper graphs) and zoosporangium formation (the lower tables) of *H. kandeliae* at different salinity (left) and temperatures (right).



Zoosporangium formation

25 C, 1 day submerg.

	0	10	20	30	40 ppt
AN-1573	+-	+	+++	++++	++
AN-1574	-	+	++	++++	+++

* aborted sporangia

30 ppt, 1 day submerg.

	8	15	20	25	30	37 C
AN-1599	-	+	+++	++++	(++)*	-
AN-1574	-	++	+++	++++	(+++)*	-

Fig. 9. Hyphal growth (the upper graphs) and zoosporangium formation (the lower tables) of *H. mycoparasitica* at different salinity (left) and temperatures (right).

influence of its hosts or other saprobic *Halophytophthora* species probably affects the occurrence of this species as mentioned above. The growth rate of this species was very low as compared to other saprobic *Halophytophthora* species. If the isolates were cultured on a medium containing a mycelial extract of *Pestalotiopsis*, however, its growth rate doubled (Nakagiri *et al*., 1998).

Host specificity

Some *Halophytophthora* species exhibit substrate-dependent or a host specific distribution. *Halophytophthora avicennae* (Gerrettson-Cornell and Simpson, 1984; Ho and Jong, 1990) has been found only on fallen leaves of *Avicennia marina* in Japanese mangroves. Since its original description in Australia (Gerrettson-Cornell and Simpson, 1984), this species has only been found on *Avicennia* leaves. A new *Halophytophthora* species has been found repeatedly, but only on fallen leaves of *Sonneratia alba* in Iriomote Island (Nakagiri, unpublished). This species is unique in having oval zoosporangia which form a long and stretching vesicle.

Conclusions

Different *Halophytophthora* species have been found to possess different growth characteristics and modes of reproduction and biology. Each species appears to be adapted to the temperature, salinity and substrate source of its habitat. Each species inhabits and occurs where and when the environmental factors in the mangrove brackish water are favourable to it. Thus, the *Halophytophthora* species show geographical, seasonal and spatial distribution in nature. It is evident that species of *Halophytophthora* are adapted to mangrove brackish water. All data shown here, however, were obtained from subtropical mangroves and from subtropical isolates. It is necessary, therefore, to examine whether the above conclusion is also true of the tropical mangroves and tropical species and strains of *Halophytophthora*. In addition, there is the possibility that undescribed species of *Halophytophthora* exist in the tropical and subtropical mangroves. Their description and characterization are important for taxonomic and ecological studies. Further studies on these can provide more information on the ecology and diversity of the *Halophytophthora* species and a better understanding of their role in the mangrove ecosystem.

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